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THE ADSORPTION OF SUGARS BY CHARCOAL.

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Since Rona and Michaelis (1910) the assumption has been generally accepted that the adsorption of sugars by charcoal is not affected to any noticeable extent by the hydrogen-ion concentration of the medium. To the mass of evidence already available in support of this view, may be added the results recently contributed by Kolthoff (1926), and Hauge and Williaman (1927). Rona and Toth (1914), and Sumner (1925) stated, however, that the presence of a certain amount of inorganic acids, like sulphuric or hydrochloric acid, showed a slight tendency to inhibit the adsorption of sugars. Bang and Bohmannson (1909), and Bohmannson (1909) noticed the complete inhibition of the adsorption of urinary sugar by concentrated hydrochloric acid. Rona and Michaelis (1908) observed, on the other hand, that glucose did not exert any lowering effect upon the surface tension of water, though it raised the tension according to the circumstances.

Contrary to the classic conception of the adsorption of sugars mentioned above, Guerrant and Salmon (1928) claimed that the adsorption of glucose by fullers' earth and norit took place more efficiently with increasing concentration of hydrogen-ion of the medium, particularly at hydrogen-ion concentration higher than 10^{-2}. This phenomenon was explained by them on the ground that the acid had a marked lowering effect on the surface tension of the glucose solution. Thus the surface tension of 1% solution of glucose was 0.5 dyne higher than that of pure water at 26° while glucose dissolved in *M* sulphuric acid solution lowered the tension of the resulting solution by 15.8 dynes at the same concentration

and temperature. These conclusions cannot be considered as substantiated in view of the consistently negative results of other investigators, much less the case where these investigators examined the adsorption of glucose in acid solutions, the pH values of which were greater than 0.87, whilst the determination of the surface tension was made on such a strong acid solution as *M* sulphuric acid. The experiments here described in the first section grew out of the difference between the results of Guerrant and Salmon, and others.

I. Influence of Hydrogen-ion Concentration of Solution on Adsorption of Glucose

The first problem to be investigated was the choice of the adsorbents. The adsorbents examined were restricted to the samples of charcoal alone, since other kinds of adsorbents proved to be less effective or ineffectual. Kahlbaum blood-charcoal was the most efficient among the charcoals tested. Next to it came, in the order named, Merck blood-charcoal, Fuji "active charcoal", norit and Merck wood-charsoal. When the efficiency of 1 gm. of Kahlbaum blood-charcoal in 100 cc. of 0.5% glucose solution is taken as the unit and expressed as 100, those of Merck blood-charcoal, Fuji "active charcoal", norit and Merck wood-charcoal are 78, 47, 28 and 4 respectively under the same conditions. Kahlbaum blood-charcoal was usd exclusively in the present experiments. The next factor investigated was the nature of the buffers. Owing to their relatively high adsorbability by charcoal, the organic compounds which are commonly used as the buffers resulted in the inhibition of the adsorption of sugar, the magnitude of which could not be ignored. The only compounds available for adjusting the hydrogen-ion concentration of the medium were inorganic acids and bases. The present investigator prepared the solution of desired pH by mixing hydrochloric acid with sodium hydroxide in the same manner as Guerrant and Salmon, and Hauge and Williaman did in their experiments. The great increase of Cl- and Na ion did not affect the sugar adsorption at all.

The reduction value of glucose (Merck's saccharum amyl-

aceum puriss. anhydricum, pro infusione) was determined by the Somogyi method (1926).

Prior to the examination of the influence of pH on the sugar adsorption several preliminary experiments were carried out. Though Rona and Michaelis (1908) proved already that the adsorption of glucose by charcoal took place instantaneously, it has been the common practice of the investigators to shake the charcoal and the sugar solution together for at least 30 minutes before its adsorptive capacity is measured. The results of the present experiments confirmed Rona and Michaelis' finding, in that the equilibrium between sugar and charcoal was attained instantaneously when 50 cc. of 1% glucose solution were shaken with more than 0.01 gm. of choalcoal. Kishimoto(1931) stated, however, that the equilibrium was set up after 36 hours when 5 cc. of 0.1 *M* glucose solution were added with 15 cc. of 0.01% suspension of charcoal, diluted to 30 cc. with water and shaken occasionally at 12°C. The influence of temperature on the sugar adsorption may be ignored, since the rise of temperature from 10° to 30°C resulted in only 1.8% inhibition of the adsorptive capacity of 1 gm. of charin 50 cc. of 1% glucose solution. The adsorptive efficiency of 0.5, 0.5, 1.0, 2.5 and 5 gm. of blood-charcoal in 50 cc. of 1% glucose solution was 18.1%, 33.0%, 64.0% and 84.7% respectively at 20°C.

The adsorption of glucose at various pH was studied in the following way. A series of solutions ranging in pH values from 0.73 to 10.06 was prepared by adding suitable quantities of sodium hydroxide to a stock solution of hydrochloric acid, the pH of which was 0.73. 0.5 gm. of glucose was dissolved in 50 cc. of these solutions and the resulting glucose solutions were shaken with 1.0 gm. of blood-charcoal for a while and filtered off. The sugar contents and pH values of the solutions were determined on aliquots before and after the adsorption. The efficiency of charcoal was also tested on glucose dissolved in distilled water, and 2*N* hydrochloric and sulphuric acid. The results are represented in Table I, together with the relative surface tension of the corresponding solutions determined by Traube's staragmometric method.

Considerable shift of pH towards the neutral point was seen

Table I.
Adsorption of Glucose at Various Hydrogen-ion Concentrations.
(50 cc. of 1% glucose solution, 1.0 gm. of blood-charcoal.)

pH of medium		Amount of sugar adsorbed (%)	Relative surface tension.
Before	After		
0.73	0.75	32.1	1.0053
1.33	1.38	32.5	1.0073
2.46	3.68	33.6	1.0059
3.74	5.72	34.2	1.0053
4.90	5.85	34.2	1.0060
6.14	6.20	34.1	1.0071
7.29	6.51	34.2	1.0070
8.83	8.47	34.0	1.0071
10.06	8.86	34.2	1.0061
Aq. dest.	With glucose	33.0	1.0050
	Without glucose	—	1.0000
$2N$ HCl	With glucose	25.2	1.0027
	Without glucose	—	1.0002
$2N$ H_2SO_4	With glucose	24.1	1.0102
	Without glucose	—	1.0101

after the adsorption. The most salient of the results is that the adsorbability of glucose did not increase under any circumstances, though there was a tendency towards its decrease in acid solution. Thus the capacity of charcoal was the same at any pH higher than 3.74, while it was inclined to become smaller with the increasing acidity of the medium beyond this value. The present investigator failed to agree with Guerrant and Salmon's observation in that the adsorption was decidedly weaker in $2N$ hydrochloric or sulphuric acid than in distilled water. The validity of the evidence was fully substantiated by the parallel examination of the surface tension of the solutions. The sequence of dissolving glucose was more an increase than a decrease of the surface tension at any acidity. It was thus clearly pointed out that whatever its actual magnitude the adsorbability of glucose tended to decrease gra-

dually in stronger acid solution. The finding of Hauge and Williaman (l. c.) will be recalled here. They insisted on the independence of the sugar adsorption from the reaction of the medium. Guerrant and Salmon tried to attribute the cause of the discord between their results and those of Hague and Williaman to the use of glucose and acid of higher concentration in their own experiments. What is then the factor that led Guerrant and Salmon, and the present investigator to such diverse results? The only difference in the conditions of the experiments was the nature of the adsorbent; they used various kinds of earth and norit, while the writer chose Kahlbaum's blood-charcoal.

II. Adsorption of Various Sugars by Charcoal.

By reference to the literature it appears that the study of the sugar adsorption has been restricted mainly to glucose. With regard to the adsorption of other sugars, much remains to be learned. The data so far available in this connection are as follows. Blake(1920) observed that the fructose fraction of invert sugar was adsorbed by bone-black in a larger amount than the glucose fraction. Miller and Bandemer (1927) stated, however, that fructose was adsorbed less than glucose by activated sugar-charcoal, norit or blood-charcoal at 80°C. These investigators noticed also the greater adsorbability of disaccharide (sucrose) than monosaccharide (glucose and fructose). The same evidence was found by Sjollema (1927, i, ii & iii; 1929).

The samples of sugars used in the present experiments were of the utmost purity available. Where satisfactory samples were not found on the market, as in the cases of galactose, mannose, xylose, arabinose, maltose and raffinose, they were put through repeated recrystallisation from alcohol or glacial acetic acid. Glucose, fructose, lactose and saccharose of sufficient purity were found in Merck's preparations. It was noticed that the reduction values of different kinds of sugars reached their own maxima after different lengths of time of heating with the Somogyi reagent.

The adsorption of monosaccharides was carried out in slightly

acid solution (pH 5±) to prevent the possible decomposition of sugars, particularly of fructose, on the surface of charcoal. Di- and other polysaccharides were, on the other hand, treated with charcoal at neutral reaction for fear of the hydrolysis in the acid medium. The adsorbability of sugars was measured after shaking 50 cc. of sugar solutions of various concentrations with 1.0 gm. of blood-charcoal.

The state of the adsorption of sugars was analogous to that of glucose; that is, the adsorption was not seriously affected by temperature and time. Raffinose was an exception. Its adsorption reached the maximum after an hour's shaking at room temperature; at the maximum point 1.8% more adsorption was observed than that after one minute's shaking. The rate of the adsorption of the sugars by charcoal is shown in Table II.

Table II.

Adsorbability of Various Sugars.

(50 cc. of sugar solutions, 1.0 gm. of blood-charcoal.)

	Concentration of sugar solution			
	0.05 *M*	0.02 *M*	0.015 *M*	0.01 *M*
	Adsorption (%)			
Arabinose	27.6	35.2	—	—
Xylose	33.1	44.0	—	—
Glucose	36.2	51.9	—	59.6
Fructose	34.2	45.0	—	52.9
Galactose	38.2	55.9	—	—
Mannose	39.3	56.3	—	—
Lactose	51.3	90.8	96.9	99.3
Maltose	—	91.8	—	99.0
Sucrose	—	88.8	—	98.7
Raffinose	40.6	89.7	98.9	100.0

The sugars divide themselves into pentose, hexose, di- and trisaccharide groups with regard to their adsorbability. Pentoses

were least adsorbed by charcoal and trisaccharide was held by the adsorbent in the largest amount. Hexoses and disaccharides came between these extremes. Rigidly interpreted, the adsorptive capacity of charcoal showed a slight variation in the sugars belonging to the same group. Thus arabinose was adsorbed less than xylose in the pentose group. In the hexose group the adsorption was most efficient in mannose and galactose. Fructose was least adsorbed. Since several samples of the purest sugars manifested always the same order of difference in their adsorbability there is no reason to assume that thi difference might be due to the seffect of the impurity contained in the samples. The adsorption of disaccharides was very much the same. Raffinose was more adsorbed than disaccharide in a dilute solution, but the order was reversed in a relatively concentrated solution. The adsorption of glycogen was very poor: It did not exceed 10% in 1% solution, while the adsorption was 96.5% for 1% raffinose solution, 78.2% for lactose, 34.3% for glucose and 22.0% for arabinose.

Freundlich's isothermal equation was found to be strictly applicable to the glucose adsorption when the concentration of the sugar was kept below 0.01*M* under the present conditions. Rona and Michaelis (1908) noticed that the glucose adsorption was not always governed by the equation. Kolthoff (1926) found that the equation was applicable to the glucose solution, the concentration of which was below 0.2*M*, when 50 cc. of the solution were treated with 3 gm. of charcoal. Kishimoto (1931) stated that the isothermal equatio nwas to be applied in the glucose solution regardless of the quantity of the adsorbent.

III. Inhibition of Sugar Adsorption by Surface-active Substances.

In the previous chapter an account was given of the difference in the adsorbability of various sugars by charcoal. Then it may be possible to forecast the variation of the inhibitory action of surface-active substances upon the adsorption of various sugars. The existing literature does not afford, it seems, sufficient evidence on the present problem. Rona and Michaelis (1908) observed

the inhibitory influence of acetone, acetic acid, etc. on the glucose adsorption. Rona and Toth (l. c.) studied the influence of urethane-derivatives on the glucose adsorption by charcoal and concluded that the depressive action of urethanes ran parallel with their adsorbability and power of lowering the surface tension. The inhibition of the glucose adsorption in the presence of ethyl alcohol was observed by Masing (1914). According to Kolthoff's investigation (l. c.) phenol is more efficient than alcohols to reduce the glucose adsorption by charcoal and much more so when hydrochloric acid is added to it.

With the object of furthering our knowledge in this direction the following experiments were set up. The surface-active substances used in the present experiments were alcohols (methyl, ethyl, prophyl & n-buthyl), acetic acid and acetone. Several series of the solutions of these substances ranging in relative surface tension from 0.97 to 0.85 were prepared. Each series consisted of the solutions of the substances of different kinds but of the same surface tension. Glucose was dissolved in these solutions in an amount of 1 gm. per 100 cc. 50 c. of the glucose solutions thus prepared were shaken with 1.0 gm. of chorcoal so as to know to what extent the adsorption of glucose might be depressed by the presence of the surface-active substances. The inhibitory action of these substances on the sugar adsorption manifested itself in a moment. The influence of temperature on the depressing power was negligible under the conditions of the experiment. The data are presented in Table III.

The depressive action of the surface-active substances was not always identical at the same surface tension. It differed according to the kinds of surface-active substances. Methylalcohol was least effective. The same degree of efficiency was found in ethyl-, propyl- and butyl-alcohol. Acetone and acetic acid, particularly the latter, reduced the glucose adsorption most remarkably. This fact may be, if not wholly, at least in part, due to the difference in the absolute amount of the substance adsorbed on the surface of charcoal. Acetone and acetic acid were found to be adsorbed in much larger quantity than alcohols.

TABLE III.

Inhibition of Glucose Adsorption by Surface-active Substances.

(0.5 gm. of glucose in 50 cc. of surface-active solution, 1.0 gm. of charcoal.)
Adsorbed amount of glucose in the absence of surface-active substance = 171.6 mg.

	Relative surface tension									
	0.97		0.95		0.925		0.90		0.85	
	Adsorption (mg.)	Inhibition (%)	Adsorption (mg.)	Inhibition (%)	Adsorption (mg.)	Inhibition (%)	Adsorption (mg.)	Inhibition (%)	Adsorption (mg.)	Inhibition (%)
Methylalcohol	156.5	8.8	146.2	14.8	133.0	22.5	118.4	31.0	92.4	46.2
Ethylalcohol	146.6	14.6	130.5	24.0	107.8	37.2	86.6	49.5	50.4	70.6
Propylalcohol	145.2	15.4	124.8	27.3	99.2	42.2	78.6	54.2	47.0	72.6
n-Butylalcohol	145.4	15.3	128.0	25.4	104.4	39.2	85.6	50.1	56.4	67.1
Acetone	128.2	25.3	99.2	42.2	71 8	58.2	55.6	67.6	30.0	82.5
Acetic acid	97.6	43.1	65.8	61.7	42.0	75.5	27.4	84.0	8.4	95.1

On comparing the inhibitory action of surface-active substances on the adsorption of various sugars, it was observed, as was expected, that the sugar which was adsorbed more was less inhibited. The inhibition was the slightest in the case of raffinose, next to which came lactose, glucose and arabinose in the order named (Table IV).

TABLE IV.

Inhibitory Action of Ethylalcohol on Adsorption of Various Sugars.
(0.5 gm. of sugars in 50 cc. of alcoholic solution, 1.0 gm. of charcoal.)

	Adsorption of sugars by 1.0 gm. of charcoal in the absence of alcohol (mg.)	Concentration of ethylalcohol			
		0.1 *M*		0.5 *M*	
		Adsorption (mg.)	Inhibition (%)	Adsorption (mg.)	Inhibition (%)
Arabinose	109.4	87.0	20.5	38.0	65.3
Glucose	171.6	142.2	17.1	72.6	57.7
Lactose	390.2	369.1	5.4	302.0	22.6
Raffinose	481.7	465.3	3.4	437.9	9.1

Kolthoff's observation (l. c.) that the addition of hydrochloric acid strengthened the inhibitory action of phenol on the sugar adsorption was disproved, to say nothing of Zuckerkandl and Messiner-Klebermass' finding (1930) that phenol was partially or completely decomposed on the surface of charcoal. The presence of 1% phenol in 50 cc. of 0.5% glucose solution reduced the adsorptive capacity of 1.0 gm. charcoal from 46.4% to 10.2%, while the capacity was depressed to 10.8% in the mixture of phenol (1%) and hydrochloric acid (0.05 *N*).

IV. Separation of Two Kinds of Sugars on the Surface of Charcoal in the Presence of Surface-active Substances.

The results so far obtained in the foregoing chapters indicate that the adsorption of the sugar which is more strongly adsorbed by charcoal, is less depressed by the presence of surface-active substances.

When a mixed solution of two kinds of sugars which have distinctly different adsorbability is treated with charcoal, the inhibitory action which these sugars may display mutually on their adsorbability should manifest itself more profoundly on the side of the sugar with less adsorbability. The effect of the surface-active substances upon the sugar with less adsorbability must also be brought out much more strikingly in this case than upon the sugar with greater adsorbability. If these assumptions are substantiated, it is highly probable that under suitable conditions the sugars may be separated on the surface of charcoal by the presence of surface-active substances.

Reviewing the literature in support of this assumption, one feels convinced of its soundness. Miller and Bandemer (l. c.) pointed out that the adsorption of glucose on charcoal was cut down by the presence of sucrose which has greater adsorbability than glucose. Pioneering studies in this line were made by Sjollema (l. c.). In his effort to know the true glucose value of blood he succeeded in separating glucose and non-glucose reducing substances (disaccharides, uric acid, creatinine, creatine, etc.) on the surface of charcoal, taking advantage of the great difference in the degree of the displacement of these compounds by surface-active substances such as acetic acid and ether. His conclusion seems, however, to need much more detailed support on physico-chemical grounds.

Glucose and sucrose were chosen as the representatives of the sugars with different adsorbability. The circumstance that sucrose belongs to the non-reducing disaccharide simplified the determinations of the experiments. The factors required for complete separation were found to be: 1) quantity of charcoal; 2) quantity of the sugars; 3) quantitative ratio of charcoal and the sugars; 4) the number of times of washing of the sugar-treated charcoal with surface-active solutions; 5) the concentration of the surface-active substances in the washing fluid.

The adsorptive capacity of 1.0 gm. of charcoal in 50 cc. of 0.01 *M* glucose and sucrose solution, and of a mixed solution which contained both glucose and sucrose in 0.01 *M* or 0.001 *M* concentration, was examined in the presence and absence of ethyl alcohol. The

concentration of alcohol was 0.1, 0.5, 1.0 and 2.0 *M*. The results are submitted in Table V.

Table V.
Adsorption of Glucose and Sucrose.
(1.0 gm. of charcoal, 50 cc. of sugar solution.)

Kind of sugar in the solution		Adsorption of sugar (%)					
		0.01 *M* sugar solution	0.001 *M* sugar solution				
				Ethyl alcohol			
		Aq. dest.	Aq. dest.	0.1 *M*	0.5 *M*	1.0 *M*	2.0 *M*
Glucose		59.6	77.0	46.6	14.8	—	—
Sucrose		98.7	100.0	100.0	100.0	—	—
Glucose + Sucrose	Glucose	33.9	73.1	45.4	13.1	3.8	0
	Sucrose	95.7	100.0	100.0	97.9	76.0	24.8

It will be observed in the table that the inhibitory influence exerted mutually by one sugar on the other manifested itself more markedly on the side of the sugar with less adsorbability. The adsorption of glucose in 0.01 *M* aqueous solution was reduced from 59.6% to 33.9% by the simultaneous presence of 0.01 *M* sucrose while the depression of sucrose adsorption caused by the presence of glucose was quite small (from 98.7% to 95.7%). The distinction in the adsorption of two kinds of sugars is revealed in a more striking manner in the presence of the surface-active substances.

It was impossible to establish conditions under which the quantitative separation of glucose from sucrose was achieved by a single treatment of the mixed solution with charcoal. The factor which reduced the glucose adsorption to zero, inhibited also the sucrose adsorption to a great extent. Herein lay the crux of the

problem. However, this difficulty was obviated by washing charcoal, according to Sjollema's initiation, with the same surface-active solution as had been used in the inhibition experiment. When 1.0 gm. of charcoal was treated with 50 cc. of alcoholic solutions which contained glucose and sucrose in the concentration of 0.001 M, the minimum number of times of washing for the complete separation of the sugars were as follows (Table VI).

Table VI.

Complete Separation of Glucose and Sucrose by Washing Charcoal after Adsorption.

(1.0 gm. of charcoal, 50 cc. of alcoholic solution, concentration of glucose and sucrose = 0.001 M.)

Concentration of alcohol (Mol)	Minimum number of times of washing	Adsorption of sugar (%)	
		Glucose	Sucrose
0.25	2	0	99.1
0.10	5	0	100
0.05	10	0	100

It may be expected that a more marked effect will be achieved when alcohol of higher concentration is used. But the concentration of alcohol was to be limited to 0.25 M at the highest, because of the disturbing influence of alcohol on the sugar determination method. Washing of charcoal with an alcoholic solution of a higher concentration than that used at the time of adsorption reduces, as a matter of course, the number of times of washing; with 0.05 M alcoholic solution as glucose solvent and 0.1 M solution as washing fluid, the number was reduced by half. The suitable concentration of alcohol was 0.05–0.1 M. The use of the alcoholic solution of relatively higher concentration carried with it the danger of reducing the tenacity of sucrose adsorption.

The data of Table VII solve the question to what extent the concentration of the sugars can be increased and to what limit the quantitative ratio of the sugars can be changed under the conditions for the complete separation of the sugars. The quantity of charcoal was 0.1 gm. and the concentration of alcohol was 0.05 M.

Table VII.
Limit of Concentration and of Quantitative Ratio of Glucose and Sucrose for Complete Separation of Sugars.
(1.0 gm. of charcoal, 50 cc. of solution.)

Concentration of sugar (Mol)		Concentration of alcohol (Mol)	Minimum number of times of washing	Adsorption of sugar (%)	
Glucose	Sucrose			Glucose	Sucrose
0.001	0.001	0.05	10	0	100.0
0.001	0.005	0.05	4	0	97.7
0.001	0.01	0.05	3	0	92.3
0.005	0.001	0.05	5	0	95.2
0.005	0.005	0.05	5	0	97.2
0.01	0.001	0.05	3	0	88.9
0.01	0.01	0.05	3	0	91.0

It is shown in the table that the concentration of either glucose or sucrose should not exceed $0.005M$; otherwise the separation is incomplete. The separation of glucose from sucrose took place more easily but with greater loss of sucrose in the case of a more concentrated sugar solution. Thus with $0.005M$ solution of the sugars 5 times washing was enough for the complete separation of glucose while with $0.001M$ solution 10 times washing was needed.

Besides ethylalcohol, acetone and acetic-acid were examined for their separating effect on glucose and sucrose. Owing to their stronger inhibitory action on the sugar adsorption, these substances were more efficient than alcohol for the present purpose (Table VIII).

The separation is by no means limited to glucose and sucrose. The principles are capable of general application so far as the difference in the adsorbability of two sugars is quite marked.

Summary.

1. Guerrant and Salmon's finding that the greater adsorption of glucose on charcoal was obtained in stronger acid solutions, was disproved. The adsorptive capacity of blood-charcoal for glucose remained the same, regardless of the reaction of the medium.

TABLE VIII.
Separation of Glucose and Sucrose in the Presence of Acetone and Acetic Acid.
(1.0 gm. of sugar, 50 cc. of solution.)

Concentration of sugars (Mol)	Surface-active substance		Minimum number of times of washing	Adsorption of sugar (%)	
	Sort	Concentration		Glucose	Sucrose
0.001	Acetone	0.05	5	0	98.5
	Acetic acid	0.05	5	0	100
0.005	Acetone	0.025	5	0	94.8
	Acetic acid	0.025	5	0	96.2

2. The adsorbability of various sugars was examined. The adsorption took place more efficiently with the sugar of a higher molecular weight.

3. The inhibitory action of surface-active substances on the sugar adsorption was studied. The inhibition manifested itself in a more striking manner in the case of the sugar with less adsorbability.

4. Under suitable conditions the complete separation of two kinds of sugars was achieved on the surface of charcoal.

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ÜBER DIE SPEZIFITÄT DER SCHLEIMHAUT-PROTEINE DES VERDAUUNGSTRAKTUS DES SCHWEINES.

Von

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Die Schleimhaut des Verdauungstraktus hat bezüglich Sekretion oder Absorption usw. in jedem ihrer einzelnen Teile eine besondere Aufgabe zu erfüllen.

Selbstverständlich spielt dabei das Nervensystem eine grosse Rolle; es ist aber leicht verständlich, dass auch die Schleimhautzellen in den einzelnen Abschnitten des Verdauungstraktus ihnen eigentümliche Eigenschaften besitzen.

Wenn dem so ist, dann erhebt sich vor uns die Frage, ob nicht auch die die Grundsubstanz der Schleimhautzellen bildenden Proteine nur ihnen eigentümliche Eigenschaften besitzen. D. h. also: In welchem Masse sind die die Zellen bildenden Proteine an deren Funktionseigentümlichkeiten beteiligt?

Es ist bereits bekannt, dass nach der Injektion von Schleimhautextrakten der verschiedenen Teile des Verdauungstraktus in den Körper durch deren spezifischen Einfluss verschiedene Vorgänge ausgelöst werden. Dagegen fehlt es noch sehr an Forschungen über die Schleimhautproteine. Ich habe deshalb aus den verschiedenen Teilen der Schleimhaut des Verdauungstraktus des Schweines hergestellte Proteine auf ihre physikalischen und chemischen Eigenschaften untersucht und festzustellen gesucht, in welchem Grade die den einzelnen Abschnitten entstammenden Proteine selbst an den oben genannten Eigentümlichkeiten der einzelnen Schleimhautabschnitte beteiligt sind.

Darstellung der Schleimhautproteine.

Der Magen, Dünndarm und Dickdarm des Schweins wurden aufgeschnitten und gut ausgespült. Danach wurde der Schleim von der Oberfläche der Schleimhaut entfernt, und die Schleimhaut von der Muskelschicht abgelöst. Hierbei wurde im Fundusteil der Magenschleimhaut die Schleimhaut von der Muskelschicht abgelöst, und das noch anhaftende Bindegewebe mit einem Rasiermesser abgetrennt. Beim Dünndarm und Dickdarm wurde die Ablösung der Schleimhaut in der Weise vorgenommen, dass der Darm auf eine Porzellanplatte ausgebreitet und durch leichtes Drücken mit einem stumpfen Messer Bindegewebe und Muskelschicht von der Schleimhaut losgelöst wurden. Die so erhaltene Schleimhaut wurde in kleine Stückchen geschnitten, in einen Leinenbeutel eingebracht, während mehrerer Stunden in fliessendem Wasser gewaschen, und dieses Waschen so lange fortgesetzt, bis auch beim Drücken des Beutels keine gelbe Flüssigkeit mehr austrat. Dann wurde der Inhalt des Beutels in ein Becherglas eingetan, mit destilliertem Wasser gut gewaschen, dann wieder in einen reinen Leinenbeutel eingetan und zur Entfernung der Flüssigkeit gut ausgedrückt. Die Schleimhautstückchen wurden dann mit reinem Seesand in einen Mörser gebracht und vollständig zu Brei zerrieben. Zu dem Brei wurde die doppelte Menge destilliertes Wasser gegeben, und darauf das Ganze 24 Stunden lang im Eisschrank stehen gelassen. Danach wurde zentrifugiert, der überstehenden Flüssigkeit die doppelte Menge absoluten Alkohols zugesetzt, der entstehende Niederschlag in eine Büschner'sche Nutsche getan, dann wiederholt mit Alkohol und Äther entfettet und getrocknet. Durch diese Behandlung entsteht aus der Magenschleimhaut ein weissliches Pulver mit gelblich-braunem Ton und aus der Dünndarm- und Dickdarmschleimhaut ein ganz weisses Pulver. Diese Pulver dienten mir zu meinen Untersuchungen.

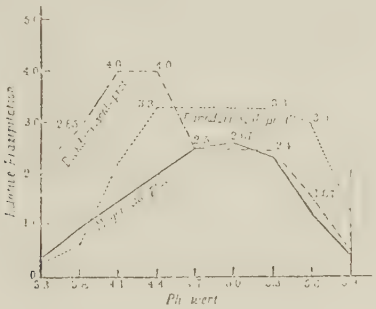
A. Physikalische Eigenschaften.

I. Über die isoelektrischen Punkte.

Da die Proteine alle bezüglich ihrer physikochemischen Natur

enge Beziehungen zu den isoelektrischen Punkten haben, ist die Bestimmung der letzteren von grösster Wichtigkeit. Zu diesem Zwecke giebt es verschiedene Methoden; ich habe die Eigenschaft des Eiweisses, bei dem isoelektrischen Punkt die geringste Löslichkeit zu zeigen, benützt, d. h. ich habe 0.05 g Eiweiss in 100 ccm N/100 Natronlauge gelöst, von der Lösung je 3 ccm auf verschiedene Ph reguliert (3.3–6.2), und zwar durch Hinzufügung von 3 ccm Acetatpuffer (1/16 Mol.) von verschiedenen Ph, darauf eine bestimmte Zeit lang stehen gelassen und die entstehende Trübung nephlometrisch bestimmt. Die Resultate sind in der Kurve 1 wiedergegeben.

Kurve 1. Isoelektr. Punkt. (Präzipitation d. Protein-Lösung in verschiedenen Wasserstoffionenkonzentrationen.)



Wie aus der Kurve hervorgeht, ist ein scharfer isoelektrischer Punkt nicht festzustellen; es ist daher vorteilhaft hier von einer isoelektrischen Zone zu sprechen. Die Werte für die verschiedenen Proteine ergeben sich daher wie folgt:

Protein der Magenschleimhaut .....Ph 4.5–5.3
Protein der Dünndarmschleimhaut .....Ph 4.4–5.3
Protein der Dickdarmschleimhaut .....Ph 4.1–4.4

II. Über die Bindungskraft mit den H-Ionen und OH-Ionen.

Zu 0.1 g des Schleimhautproteins wurden von 1/10, 1/20, 1/40, 1/80, 1/100, 1/200, 1/400 bezw. 1/800 N-Salzsäure und Natron-

lauge je 10 ccm hinzugefügt und die Mischungen während 18 Stunden im Eisschrank aufbewahrt. Darauf wurde die H-Ionenkonzentration elektrometrisch bestimmt, und mittels der Indikator-Methode nachgeprüft.

Aus der Differenz zwischen der bestimmten PH und der PH der Normallösung wurde die Bindungskraft der Protein mit der H-Ionen und OH-Ionen bestimmt.

Die Resultate sind in den Tabellen 1 und 2 wiedergegeben.

Tabelle I.
PH-Verschiebung der HCl-Lösung bei Zusatz von verschiedenen Proteinen.

Konzentration der HCl-Lösung	PH							
	N/10	N/20	N/40	N/80	N/100	N/200	N/400	N/800
+ Kein Protein.	0.943	1.235	1.58	1.904	1.98	2.388	2.618	3.041
+ Magenschl.-Prot.	0.943	1.444	1.992	3.025	3.155	3.576	4.803	5.802
+ Dünndarmschl.-Prot.	0.954	1.373	1.883	2.487	2.678	3.738	5.477	6.061
+ Dickdarmschl.-Prot.	0.943	1.332	1.824	2.423	2.603	3.738	5.223	5.971

Tabelle II.
PH-Verschiebung der NaOH-Lösung bei Zusatz von verschiedenen Proteinen.

Konzentration der NaCl-Lösung	PH							
	N/10	N/20	N/40	N/80	N/100	N/200	N/400	N/800
+ Kein Protein.	13.161	12.897	12.576	12.305	12.107	11.781	11.361	11.025
+ Magenschl.-Prot.	13.149	12.799	12.323	11.759	11.536	10.812	9.181	7.703
+ Dünndarmschl.-Prot.	13.133	12.792	12.301	11.740	11.515	10.752	9.812	8.176
+ Dickdarmschl.-Prot.	13.120	12.781	12.305	11.759	11.537	10.812	9.342	8.025

Aus der Tabelle 1 geht bemerkenswerterweise hervor, dass bei Bindung mit Salzsäure die bindende Kraft des Magenschleimhautproteins für die H-Ionen stärker ist als für die der anderen beiden Schleimhautproteinarten, wenn die Konzentration der HCl grösser als N/200 ist, während das Umgekehrte der Fall ist, wenn die Konzentration kleiner als N/200 ist. Die Bindungskraft der Dickdarm- und Dünndarmschleimhautproteine für die H-Ionen zeigt keinen allzugrossen Unterschied, doch scheint es, als ob die des Dünndarmschleimhautproteins um ein geringes stärker wäre.

Bei der Bindung der OH-Ionen, d. h. im Falle der Bindung mit der NaOH-Lösung zeigen, wie sich aus der Tabelle 2 ergibt, (eine Konzentration von N/400, d. h. einen PH von 11.3 als Grenze genommen), nach der alkalischen Seite hin alle drei Proteinarten der Schleimhäute keine grosse Differenz, bei Wendung nach der sauren Seite hin aber ist die Bindungskraft des Magenschleimhautproteins deutlich grösser als die der anderen beiden Proteinarten. Zwischen dem Protein der Dünndarmschleimhaut und dem der Dickdarmschleimhaut besteht im letzteren Falle kein grosser Unterschied, doch scheint die Bindungskraft des Dünndarmschleimhaut-Proteins für die OH-Ionen um ein geringes grösser zu sein.

Die durch das Protein gebundene Menge der H-Ionen nimmt bis zu einem gewissen Grade der Säurekonzentration allmählich zu, nach Erreichung eines gewissen Grades aber findet auch bei weiterer Erhöhung der Konzentration, was auch schon andere Autoren mit anderen Proteinen gefunden haben, keine weitere Zunahme in der Bindung der H-Ionen statt. Es ist klar, dass der genannte gewisse Grad für die verschiedenen Proteine verschieden ist.

Nach meinen Versuchen zu schliessen liegt das Optimum für die Bindung der Dünndarm- und Dickdarmschleimhautproteins mit Säure bei einem PH von 1.6, während es für das Magenschleimhautprotein bei PH 1.3 liegt.

III. Beziehungen zwischen den Proteinen und anorganischen Chloriden.

Als anorganische Chloride habe ich NaCl, KCl und $CaCl_2$

verwendet. 0.1 g Schleimhautprotein wurde mit je 10 ccm CO_2-freiem destilliertem Wasser bezw. N/10, N/100 und N/1000 Chloridlösung gemischt. Darauf wurden die Lösungen 18 Stunden im Eisschrank stehen gelassen und der PH elektrometrisch und kolorimetrisch bestimmt.

Die Ergebnisse der Versuche sind in den nachstehenden Tabellen wiedergegeben.

Wie aus den Tabellen hervorgeht, zeigt der PH-Wert bei der Mischung von Magenschleimhautprotein mit destilliertem Wasser

TABELLE III.
PH-Verschiebung von NaCl-Lösungen bei Zusatz von verschiedenen Proteinen.

Konzentration der HCl-Lösung	N/10	N/100	N/1000	Aq. dest.
+ Kein Protein.	7.01	7.01	7.01	7.01
+ Magenschleimhaut-Protein.	6.24	6.52	6.98	6.96
+ Dünndarmschleimhaut-Protein.	6.23	6.29	6.31	6.30
+ Dickdarmschleimhaut-Protein.	6.28	6.32	6.41	6.42

TABELLE IV.
PH-Verschiebung von KCl-Lösungen bei Zusatz von verschiedenen Proteinen.

Konzentration der HCl-Lösung	N/10	N/100	N/1000	Aq. dest.
+ Kein Protein.	7.01	7.01	7.01	7.01
+ Magenschleimhaut-Protein.	6.21	6.44	6.87	6.96
+ Dünndarmschleimhaut-Protein.	6.26	6.28	6.31	6.30
+ Dickdarmschleimhaut-Protein.	6.35	6.40	6.42	6.42

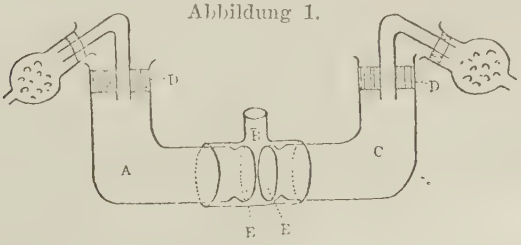
Tabelle V.
Ph-Verschiebung von $CaCl_2$-Lösungen bei Zusatz von verschiedenen Proteinen.

Konzentration der $CaCl_2$-Lösung	N/10	N/100	N/1000	Aq. dest.
+ Kein Protein.	7.01	7.01	7.01	7.01
+ Magenschleimhaut-Protein.	6.16	6.32	6.71	6.96
+ Dünndarmschleimhaut-Protein.	6.24	6.28	6.30	6.30
+ Dickdarmschleimhaut-Protein.	6.30	6.36	6.40	6.42

und der bei Zusatz von N/1000 Chloridlösung keinerlei Unterschied; wenn aber eine N/100–N/10 Chloridlösung zugesetzt wurde, dann wurde die Mischung deutlich sauer, und proportional der Konzentration der Chloridlösungen wurde auch der Ph-Wert kleiner. Bei einem Zusatz von Chlorid Lösung zu den Proteinen der Dünndarm- und Dickdarmschleimhaut bis zu der Konzentration von N/10 waren die Mischungen sämtlich sauer; der Unterschied in dem Ph-Wert der verschiedenen Kozentrationen der Chloridlösungen war aber derartig gering, dass er kolorimetrisch nicht bestimmt werden konnte.

IV. Die ungleiche Diffusion der Natrium- und Chlor-Ionen sowie auch $H^{\cdot}$- und HCO_3'-(od CO_3''-) Ionen durch eine Schicht von Schleimhautprotein.

Abbildung 1.



Der in der Figur abgebildete Apparat ist aus Glas angefertigt. Er besteht aus den 3 Glasröhren A, B und C. A und C zeigen die

gleiche Gestalt, während B drei Ausflussöffnungen hat. A und C schliessen luftdicht mit B. E und E′ sind Kollodiummembranen, durch die A und C verschlossen sind, die Entfernung zwischen E und E′ beträgt 0.3 ccm. Bei D und D′ sind die Röhren durch Röhren mit Natronkalk von der Aussenluft abgeschlossen. Die Kapazität der Röhren A und C beträgt ungefähr 10 ccm.

Versuch I.

In den Teil A des obenerwähnten Apparats wurden 5.00 ccm destilliertes Wasser und in den Teil C 5.00 ccm N/10 NaCl eingebracht, während in B eine breiartige Mischung von Schleimhautprotein und Aqua destillata eingefüllt wurde. So ist also der Inhalt der Röhre A von dem der Röhre C durch die Kolloidmembranen der beiden Röhren bei E und E′ und durch die Schleimhautprotein-Schicht in B getrennt. Dann wurden die Gummipfropfen in D und D′ eingefügt, von Zeit zu Zeit wurde der Inhalt von A und C gut mit einem Glasstab durchmischt, und dann nach 3 bezw. 6 bezw. 9, bezw. 24, bezw. 48 Stunden der Inhalt der Röhren A und C mittels der Indikatormethode auf seine $H^{\cdot}$ untersucht, weiter auch in dem Inhalt der Röhre die Chlormenge bestimmt. Als Kontrolle wurde ganz derselbe Versuch ausgeführt, nur dass in B destilliertes Wasser eingefüllt wurde.

Die Ergebnisse sind die folgenden:

Tabelle VI.
Durchlässigkeit für die Ionen bei der Magenschleimhautprotein-Schicht.

	Ph-Wert		Cl-Gehalt in 1.0 ccm (mg)	
	Aq. dest.	N/10-NaCl	Aq. dest.	N/10 NaCl
Vor der Untersuchg.	7.1	7.1	—	
Nach 3 Std.	7.1–6.8	„	—	
„ 6 „	6.8	„	—	
„ 9 „	„	„	+	
„ 24 „	6.6–5.8	7.1–7.3	0.45	2.9
„ 48 „	„	7.1	0.9	2.5

Tabelle VII.
Durchlässigkeit für die Ionen bei der Dünndarmschleimhaut-protein-Schicht.

	Ph-Wert		Cl-Gehalt in 1.0 ccm (mg)	
	Aq. dest.	N/10 NaCl	Aq. dest.	N/10-NaCl
Vor der Untersuchg.	6.8	6.8	–	
Nach 3 Std.	„	„	–	
„ 6 „	6.8–7.1	6.8–6.6	–	
„ 9 „	7.1	„	–	
„ 24 „	7.1–7.3	„	0.25	8.2
„ 48 „	„	„	0.4	3.1
„ 72 „	„	„	0.6	2,85

Tabelle VIII.
Durchlässigkeit für die Ionen bei der Dickdarmschleimhaut-protein-Schicht.

	Ph-Wert		Cl-Gehalt in 1.0 ccm (mg)	
	Aq. dest.	N/10 NaCl	Aq. dest.	N/10 NaCl
Vor der Untersuchg.	6.8–7.1	6.8–7.1	–	
Nach 3 Std.	„	„	–	
„ 6 „	„	„	–	
„ 9 „	„	„	–	
„ 24 „	6.8–7.1	6.8–7.1	0.3	3.1
„ 48 „	„		0.5	2.8

(Die doppelt unterstrichenen Werte in der Tabelle sind Annäherungswerte.)

Tabelle IX.
Kontroll-Versuch.

	Cl-Gehalt in 1.0 ccm (mg)	
	Aq. dest.	N/10 NaCl
Vor der Untersuchg.	–	
Nach 3 Std.	–	
„ 6 „	+ (schwach)	
„ 9 „	+	
„ 24 „	0.55	2.95

Aus den obengenannten Versuchen geht hervor, dass wenn Magenschleimhautprotein in die Röhre B eingebracht wurde, das destillierte Wasser sauer und die NaCl-Lösung alkalisch wurde; im Falle des Dünndarmschleimhaut-Proteins wurde das destillierte Wasser alkalisch und die NaCl-Lösung sauer, und im Falle des Dickdarmschleimhautproteins wurde ebenfalls, wenn auch nicht so deutlich, das destillierte Wasser alkalisch und die NaCl-Lösung sauer gefunden. Diese Erscheinungen müssen in ihrer Verschiedenheit zweifellos auf die Unterschiede in der Durchlässigkeit der verschiedenen Schleimhautproteine für die Na′- bezw. Cl′-Ionen zurückgeführt werden. D. h. wenn die Lösung sauer wurde, so ist das darauf zurückzuführen, dass infolge eines Überschusses an Cl′-Ionen durch Hydrolyse HCl erzeugt wurde, und wenn sie alkalisch wurde, so besagt das, dass durch einen Überschuss an Na′-Ionen durch Hydrolyse Natronlauge erzeugt wurde.

Weiter müssen wir nach allem annehmen, dass die Magenschleimhautprotein-Schicht für die Cl′-Ionen leicht, für die Na′-Ionen aber nur schwer permeabel ist, dass weiter aus diesem Grunde in dem destillierten Wasser ein Überschuss an Cl′-Ionen entsteht und die Lösung also sauer wird, entgegengesetzt aber in der NaCl-Lösung ein Überschuss an Na-Ionen, und das diese infolgedessen alkalisch wird.

Die Dünndarm- bezw. Dickdarmschleimhautprotein-Schichten dagegen sind für die Na′-Ionen leicht und für die Cl′-Ionen schwer permeabel; es entsteht deshalb in dem destillierten Wasser ein Überschuss an Na-Ionen und es bildet sich infolge Hydrolyse NaOH; die Lösung wird alkalisch, wogegen in der NaCl-Lösung durch Überschuss an Cl-Ionen sich Salzsäure bildet, und die Lösung sauer wird.

Weiter ist bei diesem Versuche bemerkenswert, dass der Chlorgehalt in dem destillierten Wasser im Falle des Magenschleimhautproteins gegenüber der Kontrolle nur einen geringen Unterschied zeigt, während im Falle des Dickdarm- und Dünndarmschleimhautproteins der Unterschied gegenüber der Kontrolle gross ist, d. h. etwa die Hälfte beträgt.

Versuch II.

Die Apparatur war dieselbe wie bei dem Versuch I. In die Röhre A wurden 5.0 ccm destilliertes Wasser, in die Röhre B Schleimhautprotein-Brei und in die Röhre C 5.0 ccm Kochsalzlösung eingebracht. Anders aber als im Versuch I wurde nun bei diesem Versuche in die Kochsalzlösung kontinuierlich Kohlensäure eingeleitet. Alsdann wurde nach 3, bezw. 6, bezw. 24, bezw. 48 Stunden in beiden Röhren der Ph-Wert nach der Indikatormethode und der Chlorgehalt bestimmt. (Die Bestimmung des Chlorgehalts geschah nach Witerhorn.) Das Kohlensäuregas wurde in einem Kipp'schen Apparat durch Einwirkung von HCl auf Marmor erzeugt und einmal durch destilliertes Wasser durchgeleitet. Als Kontrollversuch wurde unter im übrigen unveränderter Versuchsanordnung anstatt des Schleimhautproteins in die Röhre B destilliertes Wasser eingefüllt.

Die Ergebnisse sind die folgenden:

Tabelle X.
Kontroll-Versuch.

	Ph-Wert.		Cl-Gehalt in 1.0 ccm (mg)	
	Aq. dest.	N/10 NaCl + CO_2	Aq. dest.	N/10 NaCl + CO_2
Vor der Untersuchg.	7.1	7.1	—	
Nach 3 Std.	„	5.0	—	
„ 6 „	6.8	4.7–5.0	± (schwach)	
„ 24 „	6.4	5.0	0.5	2.9
„ 48 „	6.0	4.7–5.0	0.9	2.6

Aus diesen Versuchen geht hervor, dass im Falle des Magenschleimhautproteins und in dem des Dickdarmschleimhautproteins das destillierte Wasser in der Röhre A sauer wird; aber im Vergleich zur Kontrolle ist bei dem Magenschleimhautprotein die saure Reaktion stärker, während im Falle des Dickdarmschleimhautproteins eine etwas stärkere alkalische Reaktion zu beobachten ist.

Tabelle XI.
Durchlässigkeit für die Ionen bei den Magenschleimhaut-protein-Schicht.

	PH-Wert.		Cl-Gehalt in 1.0 ccm (mg)	
	Aq. dest.	N/10 NaCl + CO_2	Aq. dest.	N/10 NaCl + CO_2
Vor der Untersuchg.	6.8	7.1	—	
Nach 3 Std.	„	5.0	—	
„ 6 „	„	„	—	
„ 24 „	6.5	„	0.25	3.2
„ 48 „	6.2	„	0.35	3.1

Tabelle XII.
Durchlässigkeit für die Ionen bei der Dünndarmschleimhaut-protein-Schicht.

	PH-Wert.		Cl-Gehalt in 1.0 ccm (mg)	
	Aq. dest.	N/10 NaCl + CO_2	Aq. dest.	N/10 NaCl + CO_2
Vor der Untersuchg.	7.1	7.1		
Nach 3 Std.	„	5.0	—	
„ 6 „	6.8–7.1	„	—	
„ 24 „	6.0	„	0.35	3.05
„ 48 „	5.7	„	0.65	2.8

Tabelle XIII.
Durchlässigkeit für die Ionen bei der Dickdarmschleimhaut-protein-Schicht.

	PH-Wert.		Cl-Gehalt in 1.0 ccm (mg)	
	Aq. dest.	N/10 NaCl + CO_2	Aq. dest.	N/10 NaCl + CO_2
Vor der Untersuchg.	6.8	7.1	—	
Nach 3 Std.	„	5.3	—	
„ 6 „	6.8–7.1	5.0	—	
„ 24 „	7.1	„	0.2	3.25
„ 48 „	„	„	0.35	3.15

Der Unterschied ist aber derselbe wie im Versuch I, bei dem keine Kohlensäure zugeleitet wurde. Auch der Gehalt des destillierten Wassers an Chlor ist derselbe wie im I. Versuch. Danach ist also anzunehmen, dass die Magen- und Dickdarmschleimhautproteine gegenüber den $H^{\cdot}$-, HCO_3' oder CO_3''-Ionen eine spezifische Wirkung nicht entfalten. Der Unterschied in dem Ph-Wert scheint ebenso wie bei Versuch I auf der ungleichen Durchlässigkeit für die $Na^{\cdot}$ und Cl'-Ionen zurückzuführen zu sein.

Andererseits ist zu berücksichtigen, dass im Falle des Dünndarmschleimhautproteins das destillierte Wasser den Ph-Wert 7.1 gegenüber dem Ph-Wert 7.1–7.3 im Versuch I, der Säuregrad also eine leichte Erhöhung zeigt, im Vergleich zur Kontrolle aber stark alkalisch zu nennen ist. Diese Erscheinung kann auf keinen Fall wie in dem Versuch I mit dem Unterschied in der Durchlässigkeit für die $Na^{\cdot}$ und Cl'-Ionen erklärt werden. Das Dünndarmschleimhautprotein wirkt in spezifischer Weise den einzelnen Ionen der H_2CO_3 gegenüber. Für die HCO_3'- oder CO_3''-Ionen ist die Dünndarmschleimhautprotein-Schicht leicht permeabel, dagegen scheint sie für die H'-Ionen nur schwer permeabel zu sein. Das scheint also zu bedeuten, dass die in das destillierte Wasser übergehenden HCO_3'- oder CO_3''-Ionen, wenn sie dort im Überschuss sind, $NaHCO_3$ oder Na_2CO_3 bilden.

V. Über den Widerstand gegenüber proteolytischen Fermenten.

a) *Widerstand gegenüber Pepsin.*

Ich have mit Pepsin (Grübler) nach der Methode von Michaelis und Rotstein eine Pepsin-Standardlösung hergestellt. Als Proteinlösung diente eine Lösung von 0.05% Schleimhautprotein in N/30 HCl-Lösung, d. h. es wurden von jedem Schleimhautprotein 0.03 g in 20 ccm N/10 NaOH gelöst und sofort 40 ccm N/10 HCl hinzugefügt.

Die Bestimmung des Verdauungsgrades wurde in 2 Stufen durchgeführt.

Auf der 1. Stufe wurde eine Pepsinlösung verwendet, die in

1 ccm 20 Pepsineinheiten (nach Michaelis und Rotstein) enthielt und von da an progressiv verdünnt wurde. Hiervon wurden je 2 ccm mit je 2 ccm Proteinlösung gemischt, und 20 Minuten lang im Thermostaten auf 37°C erwärmt. Darauf wurde 1 ccm 10%iger Sulfosalizylsäure hinzugefügt und die entstehende Trübung nephelometrisch gemessen.

Auf der 2. Stufe wurden die zuletzt übrig gebliebene klare Lösung der 1. Stufe ebenso wie die zuerst erhaltene getrübte Lösung mit verschiedenen Pepsineinheiten ebenso wie auf der 1. Stufe behandelt. Da mit einer konzentrierten Pepsinlösung, die lange im Thermostat gestanden hat, bei Zusatz von Sulfosalizylsäure immer noch eine Trübung zurückbleibt, habe ich eine Lösung, die 20 Pepsineinheiten enthielt und bei 37°C 20 Minuten gewirkt hatte, und der dann Sulfosalizylsäure zugesetzt wurde, als Kontrolle mittels des Nephelometers in jedem einzelnen Falle vergleichend untersucht. Wenn darüber hinaus eine Trübung auftrat, so wurde diese als restierendes durch die angenommene Pepsineinheit nicht verdautes Protein angesehen.

Tabelle XIV.

Widerstand gegen Pepsin.

	Notwendige Min.-P.-E. um 1.0 ccm 0.05% Protein zu verdauen.	Widerstand d. Magenschleimhautproteins als 100.
Magenschleimhaut-P.	7.	100
Dünndarmschl.-P.	1.	14.3
Dickdarmschl.-P.	0.25	3.6

Das Magenschleimhautprotein zeigt den stärksten, das Dickdarmschleimhautprotein den schwächsten Widerstand.

Wie die meisten Forscher bisher angenommen haben, besitzt die Magenschleimhaut eine antipeptisch wirkende Substanz. Wie aus dem Versuch I hervorgeht, hat das Magenschleimhautprotein gegenüber dem Pepsin eine starke Widerstandskraft. Sollte das nicht darauf zurückzuführen sein, dass das sog. Antipepsin mit

dem Protein der Magenschleimhaut gemischt vorhanden ist? Um der Lösung dieser Frage näher zu kommen, habe ich die nachfolgenden Versuche angestellt, auf Grund der Tatsache, dass das Antipepsin durch Alkali zerstört wird.

1. Lösung: 0.03 g Magenschleimhautprotein wurden in 20 ccm N/10 NaOH gelöst, 24 Stunden stehen gelassen und darauf 40 ccm N/10 HCl zugesetzt. Das Ganze entspricht 0.05% Protein.

2. Lösung: 0.03 g Magenschleimhautprotein wurden in 20 ccm N/10 NaOH gelöst und darauf gleich 40 ccm N/10 HCl zugesetzt und die Lösung so sauer gemacht.

Der Widerstand der Lösungen 1 und 2 gegen Pepsin wurde wie in dem Versuch I bestimmt.

Hierbei habe ich gefunden, dass die Proteinlösungen 1 und 2 beide in gleicher Weise von 6.0 Pepsin-Einheiten verdaut wurden.

Wenn in dem Magenschleimhautprotein ein Antipepsin wirksam wäre, dann müsste es nach einer Einwirkung von NaOH während 24 Stunden, wenn auch nicht gänzlich, so doch teilweise zerstört worden sein, es müsste also in den Versuchen mit den Lösungen 1 und 2 ein Unterschied in der Verdauungswirkung bestehen. D. h. also, dass die Widerstandskraft des Magenschleimhautproteins gegen das Pepsin auf das Protein selbst, nicht aber auf das Vorhandensein eines beigemischten Antipepsins zurückzuführen ist.

b.) Widerstand gegenüber Trypsin.

Trypsin (Grübler) benützend habe ich ebenso wie im Falle des Pepsins eine Standard-Lösung hergestellt. Die Proteinlösung wurde so hergestellt, dass 0.05 g jedes Schleimhautproteins in 10 ccm N/10 NaOH gelöst und dann das Ganze mit destilliertem Wasser auf 100 ccm aufgefüllt wurde. Es entstand so eine Proteinlösung von 0.05%.

Die weitere Behandlung geschah fast genau wie bei dem entsprechenden Versuch mit Pepsin, nur dass bei Mischung von je 2 ccm Proteinlösung und Trypsinlösung 1 ccm P_H 8.0 Phosphatpuffer hinzugefügt wurde. Dann wurde während 30 Minuten bei

37°C im Thermostaten stehen gelassen und Sulfosalizylsäure hinzugefügt.

Die Ergebnisse sind die folgenden:

Tabelle XV.
Widerstand gegen Trypsin.

	Notwendige Min.-T.-E. um 1.0 ccm 0.05% Protein zu verdauen.	Widerstand d. Magenschl.-P. als 100.
Magenschl.-P.	8	100
Dünndarmschl.-P.	18	225
Dickdarmschl.-P.	12	150

Das Dünndarmschleimhautprotein zeigt den stärksten Widerstand gegen die Trypsinverdauung, darauf folgt das Dickdarmschleimhautprotein und am schwächsten ist der Widerstand des Magenschleimhautproteins.

Der Versuch wurde ausgeführt unter derselben Fragestellung wie bei dem entsprechenden Versuch mit Pepsin, d. h. er sollte feststellen, ob das Dünndarmschleimhautprotein dem Trypsin als Widerstand eine besondere Antitrypsinartige Substanz entgegenzusetzen hat. Weil aber Antitrypsin leicht durch Säure zerstört wird, wurde auch der folgende Versuch unternommen, bei dem wieder zwei Lösungen hergestellt wurden:

1. Lösung: 0.03 g Dünndarmschleimhautprotein wurden in 10 ccm N/10 NaOH gelöst, gleich darauf mit 20 ccm N/5 HCl angesäuert (N/10 HCl) und während 24 Stunden stehen gelassen. Darauf wurde wieder mit 30 ccm N/10 NaOH neutralisiert.

2. Lösung: 0.03 g Dünndarmschleimhautprotein wurden in 30 ccm N/10 NaOH gelöst und mit 30 ccm N/10 neutralisiert.

Bei diesen Versuchen habe ich gefunden, dass bei den Lösungen 1 und 2 in gleicher Weise 1 ccm 0.05%iges Protein von 17–18 Trypsin-Einheiten verdaut wurden, sodass ein Unterschied nicht zu beobachten ist. Es zeigt sich also auch hier, dass der Widerstand in dem betreffenden Protein selbst und nicht in einem besonderen Antitrypsin zu suchen ist.

B. Chemische Eigenschaften.

I. Über den Gehalt an freien Aminogruppen und Carboxylgruppen.

Zu diesen Untersuchungen habe ich je 0.1 g Schleimhautprotein verwendet.

Bei Bestimmung der Aminogruppe habe ich die Methode von Linderström (1927) benutzt. Die von dem Aceton befreite Aminogruppe wurde mit N/10 HCl in 90%iger alkoholischer Lösung titriert. Als Indikator diente Naphtylrot.

Die Carboxyl-Gruppe wurde nach Willstätter (1921) mittels Alkohol frei gemacht und mit Thymolphthalein als Indikator mittels N/10 NaOH in 90%iger alkoholischer Lösung titriert.

Die Ergebnisse sind die folgenden:

Tabelle XVI.
Gehalt an freien Amino- und Karboxyl-Gruppen.

	Notwendige Menge an N/10 HCl um die Amino-G. in 1.0 g Protein zu neutralisieren.	Amino-Index.	Notwendige Menge an N/10 NaOH um die Carboxyl-G. in 1.0 g Protein zu neutralisieren.	Carboxyl-Index.	Gesamt-N d. Proteine	NH_2 : COOH
Magenschl.-P.	7.9 cc.	11.57	10.5 cc.	8.7	12.8%	0.75
Dünndarmschl.-P.	5.6	17.34	5.7	17.04	13.6	0.98
Dickdarmschl.-P.	6.9	12.73	5.1	17.23	12.3	1.35

Wie die Tabelle 16 zeigt, kommen im Vergleich zu den anderen Schleimhautproteinen die freien Aminogruppen und die Carboxyl-Gruppen im Magenschleimhautprotein in der grössten Menge vor. Darauf folgt, was die Aminogruppe angeht, das Dickdarmschleimhautprotein und zuletzt das Dünndarmschleimhautprotein. Bezüglich der Carboxyl-Gruppe steht das Dünndarmschleimhautprotein an zweiter Stelle, während das Dickdarmschleimhautprotein an diesen Stoffen arm ist.

Ein Vergleich des Gehaltes der einzelnen Schleimhautproteine

an Stoffen der Aminogruppe und der Carboxylgruppe ergibt, wie die letzte Spalte zeigt, dass der Gehalt des Magenschleimhautproteins an der letzteren besonders reich ist, dass in dem Protein der Dünndarmschleimhaut der Gehalt an beiden fast derselbe, in dem Protein der Dickdarmschleimhaut der Gehalt an Stoffen der Aminogruppe höher als an denen der Carboxylgruppe ist. (Der absolute Gehalt des Magenschleimhautproteins an Stoffen der Aminogruppe ist höher als der der anderen Schleimhautproteine, jedoch ist der Unterschied gering, während er mit dem Gehalt der anderen Schleimhautproteine an Stoffen der Carboxylgruppe verglichen das Doppelte beträgt.)

Das von Obermeyer, Willheim (1912), Lustig (1930) u.a. betonte prozentuale Verhältnis zwischen der Amino-Gruppe und der Carboxyl-Gruppe zu dem Gesamteiweiss-Stickstoff, der sog. Amino- und Carboxyl-Index, stimmt mit dem absoluten Gehalt.

II. Über die Verteilung des Aminostickstoffs in den Schleimhautproteinen.

Die Untersuchungen wurden nach der Methode von van Slyke (1911) ausgeführt.

Die Ergebnisse sind die folgenden:

Wie die Tabelle XVII zeigt, kommt der Humin-N in dem Magenschleimhautprotein am wenigsten, in dem Dünndarmschleimhautprotein am meisten vor. Ammonia-N kommt in der Dickdarmschleimhaut in grösserer Menge vor als in den anderen Schleimhautproteinen. Die mit Phosphorwolframsäure niedergeschlagene Diamino-Säure kommt am meisten in dem Dünndarmschleimhautprotein vor, am wenigsten in dem Protein der Magenschleimhaut. (Tabelle XVIII). Unter den Diaminosäuren ist der Gehalt an Arginin-N und Cystin-N der verschiedenen Schleimhaut-Proteine ein sehr verschiedener. Arginin-N kommt in dem Dünndarmschleimhautprotein am meisten vor, in dem Magenschleimhautprotein am wenigsten. Der Gehalt des letzteren an Arginin-N beträgt nur die Hälfte von dem des ersteren. Bei dem Cystin-N verhält es sich umgekehrt. Das Magenschleimhautprotein enthält die doppelte Menge Cystin-N, verglichen mit dem Protein der

Tabelle XVII.

Amino-N Verteilung I.

		Magenschl.-P.	Dünndarmschl.-P.	Dickdarmschl.-P.
Gesamt-N als 100%	Gesamt-N	12.8%	13.6%	12.3%
	Humin-N	2.8	3.9	3.2
	Ammonia-N	10.1	10.6	13.3
Durch Phosphorwolframsäure gefällt.	Gesamt-N	27.6	31.5	29.8
	Gesamt Amino-N	14.6	15.4	14.6
	Arginin-N	2.8	7.2	4.6
	Cystin-N	0.9	0.4	0.5
	Histidin-N	16.5	16.0	10.3
	Lysin-N	7.4	7.8	7.0
Nicht gefällt.	Gesamt-N	59.5	53.7	53.3
	Gesamt Amino-N	55.3	46.3	49.0

Tabelle XVIII.

Durch Phosphorwolframsäure ausgefällt.

	Gesamt-N.	Gesamt-Amino-N.	Arginin-N.	Zystin-N.	Histidin-N.	Lysin-N.
Magenschl.-P.	100% (27.6%)	52.47%	10.2%	3.23%	59.76%	26.76%
Dünndarmschl.-P.	100% (31.5%)	48.85	22.79	1.38	51.14	24.77
Dickdarmschl.-P.	100% (29.8%)	49.05	15.63	1.78	58.76	23.72

Tabelle XIX.

Durch Phosphorwolframsäure nicht ausgefällt, und % an Humin im Verhältnis zum Gesamt-N.

	Gesamt-N.	Gesamt-Amino-N.	Nicht-Amino-N.	Humin-N. (Gesamt-N.)
Magenschl.-P.	100% (59.4%)	92.9%	7.1%	2.8%
Dünndarmschl.-P.	100% (53.7)	86.1	13.9	3.9
Dickdarmschl.-P.	100% (53.3)	91.9	8.1	3.2

Dünndarmschleimhaut. Das Protein der Dickdarmschleimhaut steht in dieser Hinsicht immer zwischen den beiden erstgenannten. (Tabelle XIX). Die mit Phosphorwolframsäure nicht ausfällbaren heterozyklischen Verbindungen, wie Prolin, Oxyprolin und Tryptophan sind im Vergleich zu dem Protein der Magenschleimhaut in dem der Dünndarmschleimhaut in doppelter Menge vorhanden. Humin entsteht bei der Hydrolyse aus einem Teile des Tryptophans. Die Tatsache, dass dieses Humin in dem Protein der Dünndarmschleimhaut in der grössten und dem der Magenschleimhaut in der geringsten Menge vorkommt, lässt darauf schliessen, dass der wirkliche Unterschied noch viel grösser ist. Auch hier steht das Protein der Dickdarmschleimhaut zwischen den beiden vorgenannten.

C. Erklärung einiger physiologischer Vorgänge auf Grund der bisherigen Resultate.

I. Über den HCl-Bildungsvorgang im Magen.

Seit 1852 Schmidt im Magen des Hundes freie Salzsäure nachwies, wissen wir, dass die saure Reaktion des Mageninhalts auf Salzsäure zurückzuführen ist. Rosemann (1921) fand, dass beim Hunde der Magensaft zu 0.56% aus Salzsäure besteht. Ob nun diese konzentrierte Säure aus dem neutral oder alkalisch reagierenden Blut oder aus den Körpersäften aufgebaut wird, ist eine sehr interessante Frage. Es ist daher nicht verwunderlich, dass sich die Forschung dieser Frage besonders angenommen hat.

Maly (1874) erklärte, dass in den Schleimhautzellen des Magens sich immer solche chemischen Vorgänge abspielten, bei denen Salzsäure gebildet wird, und zwar wie folgt:

$$NaH_2PO_4 + NaCl = Na_2HPO_4 + HCl$$
$$Na_2HPO_4 + CO_2 + H_2O = NaH_2PO_4 + NaHCO_3$$

Leo Liebermann (1891) hat aus der Magenschleimhaut eine Art Eiweiss dargestellt, von der er glaubte, dass sie für die Salzsäurebildung von der grössten Bedeutung wäre.

Koeppe (1896) hat in Tierversuchen die Ionenresorption der Magenschleimhaut festzustellen gesucht und kam zu dem Schluss,

dass das im Magensaft enthaltene Cl′ dem Kochsalz der Nahrung entstamme.

Bung (1901) hat den HCl-Bildungsvorgang im Magen mittels der Theorie der Massenwirkungen zu erklären versucht. Földes und Detre (1924) haben die Theorie der Donnanscher Membrana equilibria zur Erklärung benutzt.

Hanke, Martin, und Paul Donovan (1926) haben in dem Extrakt der Magenschleimhaut durch Hydrolyse der Alkyl-Chloride ein Salzsäure aufbauendes Ferment entdeckt und nahmen an, dass durch die Wirkung des Ferments die Magensäure-Bildung durch den nachstehenden Vorgang vor sich gehe:

$$RCl + HOH \rightarrow HCl + ROH \; ; \; NaCl + ROH \rightarrow RCl + NaOH$$

D. h. durch die Hydrolyse der Chloridester wird Salzsäure und Alkohol gebildet. Diese Chloridester werden im Blute aus Kochsalz und Alkohol aufgebaut.

Kobayashi (1927) hat verschiedenen Farbstoffe den Tieren intravenös injiziert und beobachtet, dass die Farbstoffe, die schliesslich im Magen wieder zum Vorschein kommen, sich sämtlich mit der Salzsäure verbinden und Chloride bildende alkalische organische Farbstoffe liefern und alle Verbindungen ähnlicher Formen sind. Er ist daher der Ansicht, dass derartige organische Chloride die Vorstufe zur Salzsäurebildung darstellen.

Rasser (1928) hat ebenso wie Liebermann aus der Magenschleimhaut ein in Pepsin und Salzsäure unlösliches Eiweiss dargestellt, das bei Vermischung mit physiologischer Kochsalzlösung und längerem Stehenlassen bei 37°C saure Reaktion zeigt. Der Misserfolg von Liebermann ist darauf zurückzuführen, dass er die Temperaturfrage ausser acht gelassen hat; und so konnte erst lange nach ihm Rasser nachweisen, dass dieses Eiweiss für die Salzsäurebildung von der grössten Bedeutung ist.

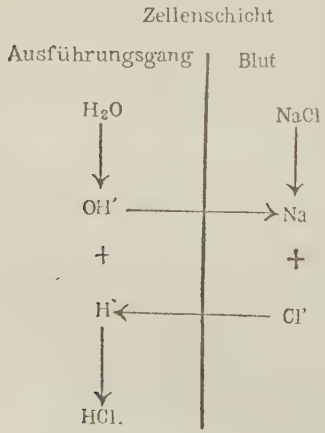
Koyama (1928) hat diese Frage mit der Ionen-Resorption durch die Magenwand erklärt, d. h. er hat die Versuche von Koeppe angewandt und sich zu der Ansicht bekannt, dass die Magenwand zwar das Na˙-Ion resorbiert, nicht aber das Cl′-Ion, dass NaCl als Molekül im Anfang abgesondert wird, zu Na˙ + Cl′ gespalten, davon das Na˙ wieder resorbiert und an seine Stelle ein H˙-Ion abgesondert

wird, und so Salzsäure entsteht.

Nach meinen Versuchsergebnissen ist bei einem PH von 2.4–11.3 die Bindungskraft des Magenschleimhautproteins für H-Ionen schwächer als die des Dünndarm- und Dickdarmschleimhautproteins, wogegen die für OH-Ionen sehr stark ist. Wenn man das in Berücksichtigung zieht, dann muss man auf den Gedanken kommen, dass wenn Protein mit einer Chloridlösung gemischt wird, mehr als die anderen Schleimhautproteine das Magenschleimhautprotein besonders saure Reaktion zeigt, was auf den nachstehenden Vorgang zurückzuführen sein wird. Im Falle von NaCl ist nach der Gleichung $NaCl + H_2O \rightarrow NaOH + HCl$, der Mengenanteil des NaCl sehr klein; doch ist anzunehmen, dass sich NaOH und HCl gebildet haben. Von diesen wird sich die NaOH mit dem Magenschleimhautprotein verbinden, während HCl im Überschuss bleibt, und so eine schwach saure Reaktion entsteht.

Das Magenschleimhautprotein hat gegenüber den anorganischen Chloriden eine grosse Bindungskraft mit den Kationen, doch ist die zu den Anionen nur gering. Was nun die Durchlässigkeit der Proteinmembran für Ionen angeht, so zeigt es sich, dass das Magenschleimhautprotein für die Cl-Ionen leicht, für die Na-Ionen schwer permeabel ist. Die Eigenschaften des Proteins sind je nach der Natur der das Protein bildenden Aminosäuren selbstverständlich verschieden. Nach meinen Versuchen enthält das Magenschleimhautprotein mehr Carboxyl als die Darmschleimhautproteine, aber der Gehalt an Hexon-Basen ist bei dem ersteren gering, welche Tatsache mit den physikalischen Eigenschaften des Magenschleimhautproteins gut übereinstimmt.

Abbildung 2.



Es ist klar, dass die Eigenschaften des Proteins, das den Hauptkomponenten der Schleimhautzellen

darstellt, auch die Eigenschaften dieser Zellen sind.

Die Zellen besitzen daher vielleicht die Eigenschaften einer semipermeablen Membran und sind gegenüber den anorganischen Chloriden des Blutes für die Cl-Ionen leicht, aber für die Kationen ($Na^{\cdot}$, $K^{\cdot}$ usw.) schwer permeabel.

Bei einem Vorgang, wie ihn die Abbildung 2 zeigt, werden die Cl'-Ionen aus dem Blut die Zellenschicht passieren, in den Ausführungsgang der Drüsen eindringen, und hier einer Wasserhydrolyse ausgesetzt, wobei HCl gebildet wird. Diese Salzsäure wird dann wohl in den Magen abgesondert werden. Das durch die Hydrolyse entstehenden OH-Ionen wird sich mit den $R^{\cdot}$ (Kationen $Na^{\cdot}$, $K^{\cdot}$ usw.) verbinden und als ROH in die Blutbahn übergehen.

Nach Krammer ist das Na im Blut normalerweise in einer Konzentration von 0.13% vorhanden. Von diesem besteht 3/4 als NaCl. das im Blute in einer Konzentration 0.6–0.67% vorhanden sein soll. Kalium- und Calciumchloride sind zwar auch vorhanden, aber im Vergleich zum NaCl nur in geringen Mengen, d. i. etwa 1/20 oder 1/30. Wenn man das berücksichtigt, so muss man wohl oder übel zu der Annahme kommen, dass die Salzsäure des Magensaftes in erster Linie von dem NaCl des Blutes hergestellt wird.

Nun sind aber auch in dem reinen Magensaft kleine Mengen Na und K vorhanden, was darauf zurückzuführen sein wird, dass die Impermeabilität der Magenschleimhaut für die Kationen keine absolute ist; es werden mit den Cl-Ionen auch $K^{\cdot}$, $Na^{\cdot}$ usw.-Ionen die Zellen passieren und in das Magenlumen eindringen. Und wenn dem so ist, so wird der Menge der passierenden Kationen entsprechend, die Zahl der durch Hydrolyse in HCl umgewandelten Cl-Ionen geringer werden müssen. Im wirklich reinen Magensaft wird sich die Chlormenge nie verändern, aber ihre Verteilung auf Salzsäure und Chloride wird zeitlichen Schwankungen unterworfen sein, wie das schon von vielen Forschern nachgewiesen worden ist. Auch ich habe an dem Pawlow'schen isolierten Kleinmagen nachgewiesen, dass die Salzsäuremenge des Magensaftes zeitlichen Veränderungen unterworfen ist, die Chlormenge dagegen immer konstant bleibt.

II. Über den Ursprung der Alkalität des Darmsafts.

Bezüglich der P_H des Darminhalts besitzen wir viele Berichte; die verschiedenen Versuchsresultate stimmen aber leider nicht überein.

Dass die Reaktion des reinen Darmsaftes immer alkalisch ist, haben bereits Thiry, Villa u. a. mit Sicherheit nachgewiesen. Über die Vorgänge bei der Sekretion des Darmsaftes haben wir viele Theorien, die sie mit dem Nervensystem in Verbindung bringen, aber auch die humorale Theorie. Ungeachtet der verschiedenen Arbeiten auf diesem Gebiete sind Forschungen auf physikalischen und chemischen Gebiete aber doch verhältnismässig selten.

Heidenhain hat in eine Darmschlinge Kochsalzlösung eingebracht und nach einiger Zeit gefunden, dass die Lösung alkalisch geworden war, und dass darin mehr noch als im reinen Darmsaft Na_2CO_3 enthalten war.

Oshima (1925) fand, dass bei Einfüllen des schwer resorbierbaren $MgSO_4$ als isotonische Kochsalzlösung in die lebende Darmschlinge, die Konzentration des NaCl keine Rolle spiele, da das NaCl immer von der Darmwand resorbiert werde, und die Darmschleimhaut eine irreziproke Permeabilität zeigt. Danach hat Matsumoto (1928) die Versuche von Oshima nachgeprüft und gefunden, dass wenn bei Anwendung einer durch $MgSO_4$ isotonisch gemachten NaCl-Lösung die Konzentration der letzteren geringer als die im Blute ist, das Kochsalz im Gegenteil aus dem Blute in das Darmlumen sezerniert wird. Weiter fand er, dass bei Lähmung der Darmsekretion durch Atropin, Morphin und Äther, selbst wenn die NaCl-Konzentration im Darm geringer ist als im Blute, erst das NaCl von der Darmwand resorbiert wird.

Oyama (1928) füllte in die isolierte Darmschlinge anorganische Chloride usw. und bestimmte die Resorptionsgeschwindigkeit, wobei er fand, dass das Na-Ion nur schwer resorbierbar ist. Er füllte dann Lösungen ein, die kein Na-Ion enthielten und fand, dass dann in solchen Lösungen Na-Ionen auftreten, was er damit erklärte, dass die Darmwand die Eigenschaft einer einseitigen

Durchlässigkeit besitze. Weiter erklärte er die Tatsache, dass der Darmsaft alkalisch ist, damit, dass das mit der Nahrung aufgenommene und von der Blutbahn ausgesonderte NaCl in $Na^{\cdot} + Cl'$ gespalten wird; das Cl' wird dann leicht resorbiert, während das mit dem Na' nicht der Fall ist, das sich vielmehr mit dem im Darm vorhandenen oder aus dem Blut leicht sezernierten HCO_3' zu $NaHCO_3$ verbindet.

Aus diesen und meinen eigenen Versuchsergebnissen muss ich annehmen, dass es sich bei der Resorption und Sekretion durch die Darmwand um gänzlich verschiedene Dinge handelt. Die Resultate der oben erwähnten Experimente (Matsumoto, Oyama u.s.w.) sind leicht dadurch zu erklären, dass zu gleicher Zeit einerseits die Resorption der Cl- und Na-Ionen erfolgt und andererseits die Na-Ionen abgesondert werden.

Aus meinen Versuchsresultaten geht hervor, dass die Darmschleimhautproteinschicht für die Na-Ionen leicht passierbar ist, dagegen nicht für die Cl'-Ionen. Weiter habe ich gefunden, dass die Dünndarmschleimhautproteinschicht besonders bei H_2CO_3 die H-Ionen nur schwer passieren, dagegen die HCO_3'- und CO_3-Ionen sehr leicht.

Da das Protein solche Eigenschaften besitzt, ist anzunehmen, dass auch die Schleimhautzellen dieselben Eigenschaften besitzen. D. h. die Schleimhautzellen des Darms besitzen die eigentümliche Eigenschaft einer semipermeablen Membran, die gegenüber dem Kochsalz im Blut für die Na-Ionen leicht, für die Cl-Ionen schwer passierbar ist. Weiter sind die Dünndarmschleimhautzellen gegenüber dem H_2CO_3 des Blutes für das H-Ion schwer, dagegen für HCO_4' und CO_3'' leicht permeabel.

Wie aus den Abbildungen 3 und 4 hervorgeht, ist im Dünndarm der Darmsaft wahrscheinlich alkalisch. Na-Ionen, die die Zellenschicht passiert haben, werden in den Ausführungsgängen der Drüsen z. T. zu NaOH durch Wasserhydrolyse, ein Teil aber verbindet sich mit den zusammen ausgetretenen HCO_3 oder CO_3-Ionen zu $NaHCO_3$ oder Na_2CO_3, und der Darminhalt wird somit alkalisch. Im Dickdarm werden die im Ausführungsgang der Drüsen einer Hydrolyse unterworfenen Na-Ionen zu NaOH gebunden und nur

Abbildung 3.

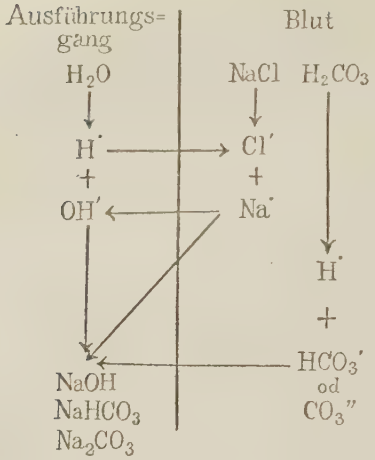
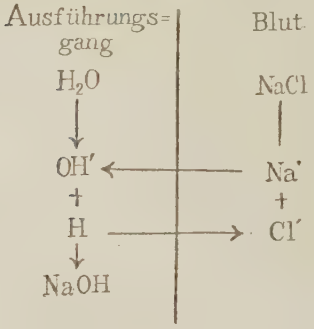


Abbildung 4.



durch dieses das Sekret alkalisch.

III. Die Ursache dafür, dass die Magenwand von dem Magensaft nicht verdaut wird.

Dass im physiologischen Zustande die Magenwand durch die starke Verdauungswirkung des Magensaftes nicht verdaut wird, ist eine sehr interessante Tatsache, die seit alters von den Autoren untersucht, und doch immer noch nicht völlig erklärt worden ist.

Zuerst hat Hunter (1872) bei eingegangenen Tieren beobachtet, dass ein Teil der Magenwand durch den eigenen Magensaft verdaut worden war, doch schloss er, dass das lebende Gewebe nicht der Verdauung durch den eigenen Organismus unterworfen werden könnte. Danach haben viele Forscher (Claude Bernard, Pavy, Viola, Contejean, Katzensteiner u.a.m.) durch Tierversuche den direkten Beweis für diese Tatsache erbringen wollen, indem sie ohne Blutkreislaufstörungen Organe und Gewebe usw. implantierten. Doch sei es nun dass die angewendeten Methoden verschiedene waren oder Abweichungen in den verschiedenen zu den Versuchen gebrauchten Organen bestanden, jedenfalls stimmen die verschiedenen Versuchsergebnisse nicht überein.

Ob nun wie Hunter behauptet hat, lebendes Gewebe verdaut werden kann, oder nicht, steht also noch in Frage, dass aber die Magenwand gegenüber dem Magensaft eine starke Widerstandskraft besitzt, das steht ausser aller Frage.

Man hat die Unverdaulichkeit der Magenwand auf verschiedenste Art und Weise erklären wollen, aber von allen Annahmen ist wohl die am wahrscheinlichsten, dass die wahre Ursache in der Schutzwirkung des Schleims und dem Vorhandensein von Antipepsin liegt.

Thiry (1864) fand, dass künstlicher Magensaft eine grössere Reizwirkung besitzt als natürlicher, und nahm daher an, dass in dem natürlichen Magensaft Schleim enthalten sei.

Harly (1864) hat der Nahrung von Kaninchen Schleim beigemischt und gefunden, dass diese Nahrung schlechter verdaut wurde, als wenn kein Schleim beigemischt worden war.

Kaufmann (1906) berichtete, dass bei Kranken mit Magengeschwür der Schleim in dem Magensafte fehlte.

Bezüglich des Antipepsins hat schon 1891 Frenzel behauptet, dass in der Magenschleimhaut ein Antipepsin vorhanden sei, das die Verdauung der Magenwand durch die Magensäure verhindere. Das Verdienst aber, das Antipepsin zuerst künstlich aus der Magenschleimhaut dargestellt zu haben, gebührt Danilew (1901).

Danach haben in langer Folge viele Forscher, so Blum (1906), Weinland (1903), Hensel (1903) u.a. das Antipepsin nicht nur aus der Magenschleimhaut, sondern auch aus Darmparasiten, Magenwand, Leber, Milz, Herzmuskeln, Darm, Nieren, Serum usw. isoliert.

Katzenstein (1912) und auch Isobe (1915) haben die Beziehungen zwischen Magenschleimhaut-Antipepsin und der Verdauungskraft des Magensaftes untersucht, doch sind sie nicht zu ausreichenden Resultaten gekommen. Ookuro und auch Lieblein (1913) haben bei an Magengeschwür Erkrankten den Antipepsingehalt des Serums bestimmt und gefunden, dass zwischen letzterem und dem Geschwür Beziehungen bestehen, weiter aber sind auch sie nicht bei diesen Untersuchungen gekommen.

Nach meinen Versuchsresultaten sind sowohl das Protein der

Magenschleimhaut, als auch das der Dünndarm- und der Dickdarmschleimhaut gegen Pepsin sehr widerstandsfähig. Und diese Widerstandskraft ist m. E. auf die Proteine selbst, nicht auf die Beimischung von Antipepsin zurückzuführen. Das Protein bildet die Grundsubstanz der Zellen, es ist daher wohl zu vermuten, dass auch die Schleimhautzellen dieselben Eigenschaften besitzen wie das Protein. Es ist daher auch anzunehmen, dass die Magenschleimhautzellen eine stärkere Widerstandskraft gegen das Pepsin zeigen, als z.B. die Darmschleimhauptzellen u.a.m. Dass im physiologischen Zustande die Magenwand nicht vom Magensaft verdaut wird, ist selbst wenn die Schutzwirkung des Schleims und die Gegenwart von Antipepsin mitwirken, auf den Widerstand der Zellen selbst gegen das Pepsin als eine der wichtigsten Ursachen zurückzuführen.

Zusammenfassung.

1. Die isoelektrischen Punkte der Schleimhautproteine des Schweines sind die folgenden: Protein der Magenschleimhaut PH 4.5–5.3, Protein der Dünndarmschleimhaut PH 4.4–5.3, und Protein der Dickdarmschleimhaut PH 4.1–4.4.

2. Bei Bindung mit Salzsäure ist die Bindungskraft des Magenschleimhautproteins für die H-Ionen kleiner als die der anderen beiden Schleimhautproteins, wenn die HCl-Konzentration $<N/200$ ist.

Bei Bindung mit Natronlauge, deren Konzentration $<N/400$ ist, ist dagegen die Bindungskraft des Magenschleimhautproteins für die OH-Ionen viel grösser als die der anderen beiden Proteine.

3. Bei jeder Mischung mit anorganischen Chloridlösungen wird das Magenschleimhautprotein zwar geringgradig doch sicher angesäuert, und zwar parallel zu der Chloridkonzentration, was bei den anderen Proteinarten nicht der Fall ist.

4. Das Magenschleimhautprotein ist für die Cl'-Ionen leicht, aber für die Na'-Ionen schwer permeabel, während das Dünndarm- und Dickdarmschleimhautprotein in Gegensatz dazu stehen. Das Dünndarmschleimhautprotein ist für die H'-Ionen schwer, aber für HCO_3'- oder CO_3''-Ionen leicht permeabel, das Magen- und Dick-

darmschleimhautprotein zeigen dagegen für dieselben Ionen keine entscheidende Differenz in der Permeabilität.

5. Gegen Pepsin ist das Magenschleimhautprotein am stärksten widerstandsfähig; ihm folgt das Dünndarmschleimhautprotein, während das Dickdarmschleimhautprotein in dieser Hinsicht am schwächsten ist. Die Widerstandskraft des Magenschleimhautproteins ist nicht auf das Vorhandensein eines beigemischen Antipepsins, sondern auf das Protein selbst zurückzuführen.

Gegen Trypsin zeigt das Dünndarmschleimhautprotein den stärksten Widerstand, ihm folgt das Dickdarmschleimhautprotein, während das Magenschleimhautprotein am wenigsten resistent ist; die Widerstandskraft des Dünndarmschleimhautproteins beruht nicht auf einem Antitrypsin, sondern auf seiner Beschaffenheit selbst.

6. Das Magenschleimhautprotein enthält mehr freie Aminogruppen und Carboxylgruppen als das Dünndarm- und Dickdarmschleimhautprotein. Die Carboxylgruppen sind hierbei doppelt so zahlreich, während die Menge der enthaltenen freien Aminogruppen bei den drei Proteinarten nur wenig Unterschiede zeigt.

7. In Berücksichtigung der Verteilung des Aminostickstoffs der drei Schleimhautproteine konstatiert man den grössten Unterschied des Gehaltes zwischen dem Arginin-N und dem Cystin-N; am meisten kommt der Arginin-N im Dünndarmschleimhautprotein vor, am wenigsten im Magenschleimhautprotein, und zwar im ersteren doppelt so viel als im letzteren. Bei dem Cystin-N verhält es sich umgekehrt.

Die Differenz der Gehaltmenge an anderen Amino-N, welche jede der drei Proteinarten enthält, ist nur gering. Das Dickdarmschleimhautprotein steht zwischen dem Magen- und Dünndarmschleimhautprotein bezgl. des Gehalts an den meisten Amino-N und zwar mit nur einigen Ausnahmen.

8. Zur Erklärung der HCl-Bildung der Magenschleimhaut ist aus den Resultaten der oben erwähnten sowohl chemischen als physikalischen Experimente zu schliessen, dass die Zellen Magenschleimhaut vielleicht die Eigenschaften einer semipermeablen Membran besitzen und gegenüber den anorganischen Cloriden des

Blutes für die Cl-Ionen leicht und für die Kationen ($Na^{\cdot}$, $K^{\cdot}$, u.s.w.) schwer permeabel sind. Bei einem Vorgang, wie die Abbildung 2 ihn zeigt, passieren die Cl'-Ionen aus dem Blut die Zellenschicht, dringen in den Ausführungsgang der Drüsen ein, sind hier einer Wasserhydrolyse ausgesetzt und bilden dabei HCl; diese Salzsäure wird wohl in den Magen abgesondert.

9. Weiter ist wohl die Vermutung zulässig, dass auch die Schleimhautzellen des Darmes die Eigenschaft einer semipermeablen Membran, die gegenüber dem Kochsalz im Blut für $Na^{\cdot}$Ionen leicht und für die Cl'-Ionen schwer passierbar ist, besitzen, und dass die Dünndarmschleimhautzellen gegenüber dem H_2CO_3 des Blutes, für die $H^{\cdot}$-Ionen schwer, dagegen für die HCO'_3- od CO''_3-Ionen leicht permeabel sind; die $Na^{\cdot}$-Ionen, die die Zellenschicht des Dünndarmes passiert haben, werden in dem Ausführungsgang der Drüsen z. T. zu NaOH durch Wasserhydrolyse synthetisiert; ein Teil verbindet sich mit den zusammen ausgetretenen HCO'_3- oder CO_3''-Ionen zu $NaHCO_3$ oder Na_2CO_3, und der Darminhalt wird somit alkalisch.

Im Dickdarm werden im Ausführungsgang der Drüsen einer Hydrolyse unterworfene $Na^{\cdot}$-Ionen zu NaOH gebunden, und nur durch diese das Sekret alkalisch.

10. Dass im physiologischen Zustande die Magenwand nicht von dem Magensaft verdaut wird, ist auf den Widerstand der Zellen selbst, die hauptsächlich aus dem gegen das Pepsin stark resistenten Protein bestehen, eben so gut als auf andere Faktoren zurückzuführen.

Am Schlusse dieser Arbeit ist es mir aufrichtiges Bedürfnis Herrn Prof. Dr. K. Kodama und Merrn Prof. Dr. S. Goto, meiner hochverehrten Lehrern, für die gütige Anregung zu dieser Arbeit, sowie ständige Leitung und vor allem auch für die freundliche Durchsicht meines Manuskriptes meinen herzlichsten Dank auszusprechen.

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STUDIES ON EXSICCOSE.

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Introduction.

One of the most pressing problems in paediatrics is to get a clearer insight into the nature of infant toxicosis and to lessen the mortality as much as possible.

As to the etiological aspect of toxicosis in dyspepsia a theory was advanced by many investigators [Ad. Czerny, 1894; P. Heim and K. John, 1910; W. McKim Marriott, 1920; F. Göppert, 1920; G. Bessau and S. Rosenbaum, 1928] that the exsiccose is the real and the sole cause of this symptom, though some others maintained the opposite idea.

Quite recently E. Shiff (1929) has made a valuable contribution in favour of this exsiccose theory. According to him, reduction of water supply to the amount of 40-50 percentage of the daily dose caused a severe intoxication due to some abnormal cleavage of protein.

The past two years the author also studied the elucidation of intoxication symptoms of exsiccose, the results of which are given in this communication.

I. The Influence of Nutritive Materials on Exsiccose.

From the theoretical as well as the therapeutical point of view it seems quite important to investigate the effects of various diets on the intensity of intoxication when the animal has been subjected to exsiccose.

In this respect, E. Kramár (1926) has already noticed in his experiment that infants nourished on a protein-free diet endure

water deficiency well for several days, while those nourished with a sufficient amount of protein manifest severe symptoms of toxisosis.

Since this fact is of primary importance for the treatment of exsiccose, the author repeated the experiment with full confirmation. At the same time the experiment was extended to various sorts of diets and all of the results obtained are reported in this communication.

Experiments.

Throughout the experiments young healthy albino rats, weighing approximately 30 gm., were used.

They were divided into seven groups, each consisting of five rats, and were fed on different diets, as is indicated in Table I. Water was given ad libitum.

Table I.
The composition of the diets.

	Basal diet	Diet excess in protein	Diet excess in carbohydrate	Diet excess in fat	Diet deficient in salt mixture
Wheat	80.4	25.4	85.4	40.4	80.4
Casein	10.0	65.0	5.0	10.0	10.0
Butter	5.0	5.0	5.0	5.0	5.0
Lard	—	—	—	40.0	—
Salt mixture (McCollum No. 318)	3.6	3.6	3.6	3.6	—
Vitamin B (oryzanin)	1.0	1.0	1.0	1.0	1.0

	Diet deficient in Vitamin A	Diet deficient in Vitamin B
Polished rice	80.4	80.4
Casein	10.0	10.0
Butter	—	5.0
Lard	5.0	—
Salt mixture (McCollum No. 318)	3.6	3.6
Vitamin B (oryzanin)	1.0	—

The water content of each diet was determined and is recorded in Table II.

No appreciable difference was observed among them.

Table II.
The content of water of the diets.

	Content of water %
Basal diet	7.7
Diet excess in protein	9.4
Diet excess in carbohydrate	7.7
Diet excess in fat	6.2
Diet deficient in salt mixture	6.3
Diet dificient in Vitamin A	7.2
Diet dificient in Vitamin B	6.9

At nine o'clock every morning the body weight, the quantity of water and diet consumed the day before, were noted and new materials were supplied.

After three weeks of nourishment on this diet, water was eliminated from the diet and the observation was continued until the animals died. All the results are illustrated in the following diagrams (Fig. 1, 2, 3, 4, 5, 6).

In comparing the growth of each diet group, those fed on the basal diet and on the diet with excess of carbohydrate showed a most favourable growth; while those fed on a diet excess in butter, deficient in salt mixture and in vitamin B. showed retarded development. When the water was eliminated from the diet, all the groups suffered a sudden decrease in weight. This was more particularly pronounced in those fed on the diet excess in protein and lacking in the salt mixture. The group on a diet excess in butter showed, however, somewhat different behavior; namely the growth was rather retarded when the water supply was sufficient, but showed more resistance against the exsiccose.

As to the quantity of food, the groups nourished on the basal diet and diet with an excess of carbohydrate consumed a larger quantity than others, and those on diets with excess of butter or deficient in vitamin B, much less.

After the elimination of water, the groups fed on diets with

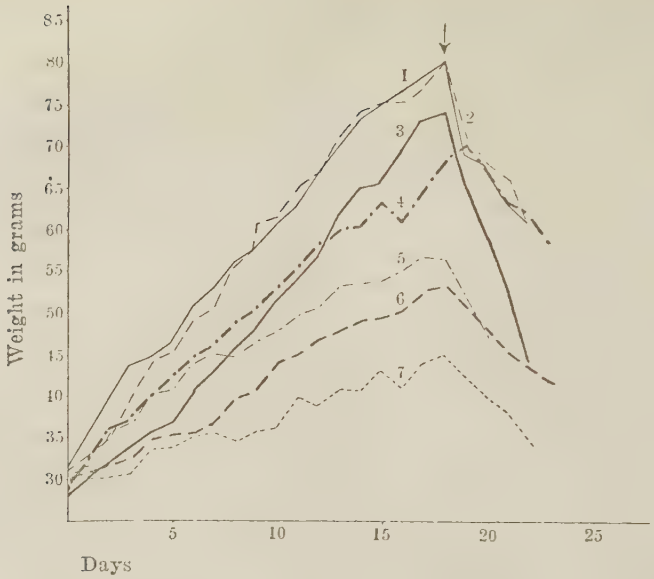

Figure 1. Curves showing the growth of rats.
(Each curve is the average for five rats).

——————— Basal Diet group
— — — — Excess in Carbohydrate diet group
——————— Excess in Protein diet group
— — — — Excess in Fat diet group
—·—·—·— Deficient in Salt mixture diet group
—— · —— · —— Deficient in Vitamin A diet group
---------- Deficient in Vitamin B diet group
↓ Mark shows where water was eliminated.

excess of protein and deficient in the salt mixture lost their appetites.

The significant difference in the curves of the water consumed is noteworthy. It is true that the group, nourished on the diet excessive in protein, drinks larger quantities of water than the others. The average of the surviving interval of rats of each group after the water was eliminated was noted also and illustrated in Fig. 7. To give evidence that the results of the above experiment has nothing to do with the individuality of the animal, a similar experiment was repeated on the litters, the result of which is illustrated in Fig. 8.

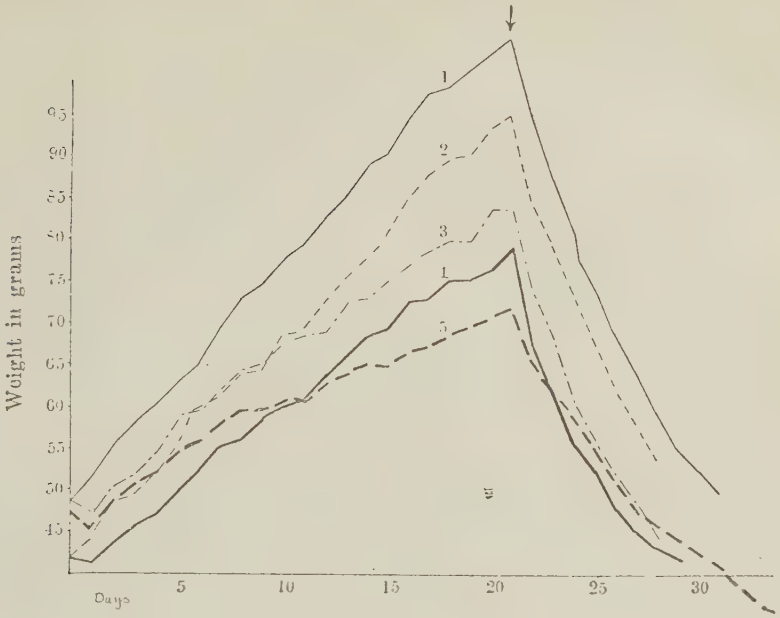

Figure 2. Curves showing the growth of litter in each diet group.

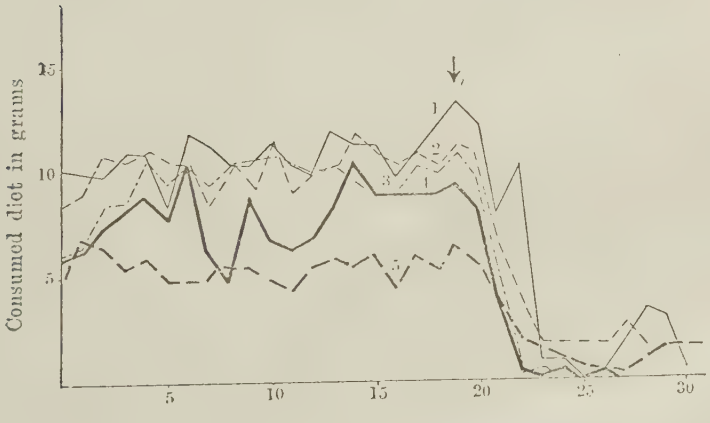

Figure 3. Curves showing the quantity of diet consumed in 24 hours by litter.

↓ Mark shows where water was eliminated.

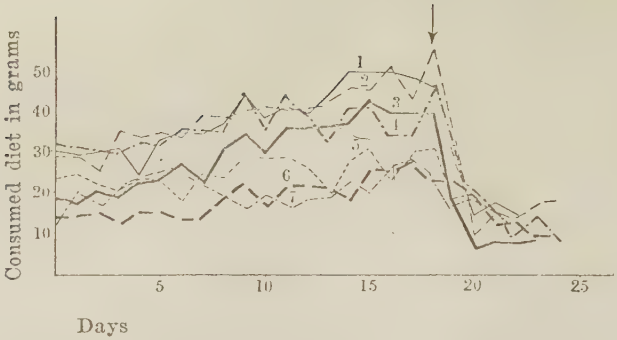

Figure 4. Curves showing the quantity of diet consumed in 24 hours by five rats.

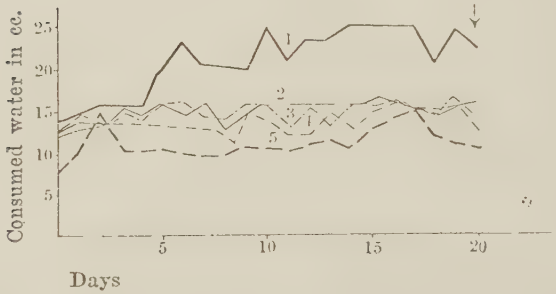

Figure 5. Curves showing the quantity of water consumed by litter in 24 hours.

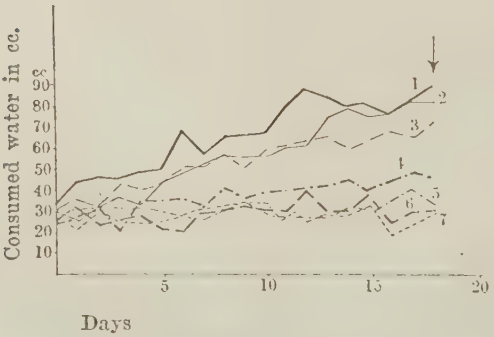

Figure 6. Curves showing the quantity of water consumed by five rats in 24 hours.

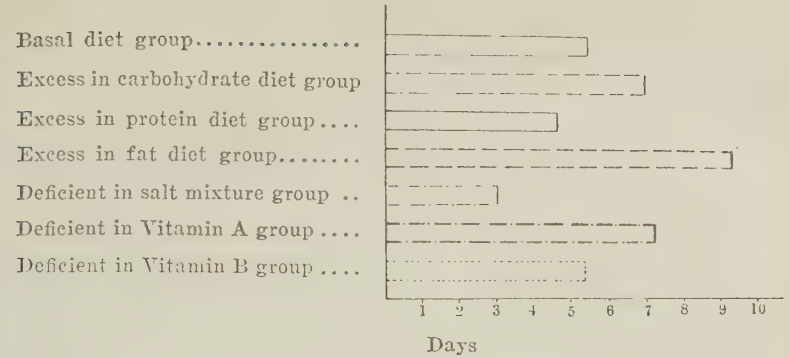

Figure 7. The effect of various diets on the average interval of survival of five rats when water was eliminated completely from the diet.

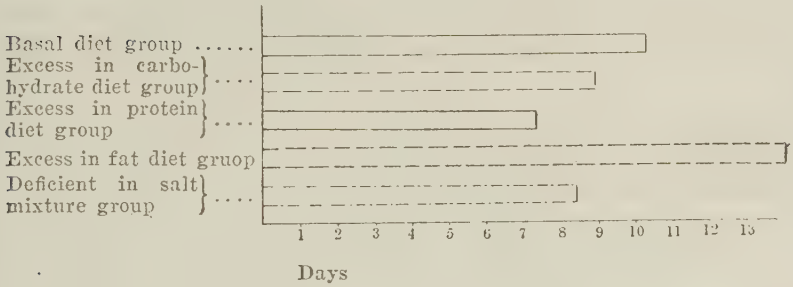

Figure 8. The effect of various diets on the surviving interval of the litter when water was eliminated from the diet.

From the above figures it is obvious that the group nourished on the diet deficient in the salt mixture and excess in protein exhabited the shortest duration of life, and next the one fed on the diet deficient in Vitamin B, while the group fed on the diet with an excess of fat survived comparatively longer.

Discussion.

The foregoing experiments reveal clearly that the group fed on a diet with an excess of protein is quite distinct in two respects; namely, in the abundant consumption of water and in the least resistance to exsiccose. The explanation may be sought in the

excess formation of urea from protein which requires much water to be dissolved and excreted into urine.

In this connection reference may be made to Voit's experiment upon dogs which shows that when urea was added to a normal diet a much larger amount of water than usual was needed.

Further W. Straub (1899) recognized in his experiment that the protein metabolism was much accelerated in exsiccose.

Taking these facts into consideration, we are justified in assuming as Schiff has already referred to, that exsiccose develops most intensely in case of a protein diet.

The reasons why the animal fed on a diet deficient in the salt mixture proved to have little resistance to exsiccose may be reduced to the fact that, owing to the decrease of its salt contents the tissue loses the power to retain the water osmotically.

The following data proves this fact in a convincing manner.

Table III.

Water content of the tissues of rats nourished on diets of various nutritive materials. (The rats were killed four days after the water was taken from their diet.)

	Basal diet			Diet excess in carbohydrate			Diet excess in protein			Diet excess in fat			Diet deficient in saltmixture		
	Ex.	Normal	Difference	Ex.	Normal	Difference	Ex.	Normal	Difference	Ex.	Normal	Difference	Ex.	Normal	Difference
Liver	29.4	30.3	0.9	28.9	30.3	1.4	29.1	30.3	1.2	28.8	30.2	1.4	28.0	30.4	2.4
Muscle	24.4	26.6	2.2	24.7	26.6	1.9	24.2	25.7	1.5	24.5	27.1	2.6	24.1	27.0	2.9
Heart	24.5	25.9	1.4	26.2	27.9	1.7	24.7	26.5	1.8	24.4	26.9	1.5	24.0	26.7	2.7

The reason why the group fed on a diet excess in fat showed greater resistance to exsiccose is quite unknown. It may be suggested however that the quantity of water produced by oxidation of fat is greater than that of any other nutritive material, as the following Table IV, shows:

Table IV.
Quantity of water produced by oxidation.

100 g. Fat	107.1 g.
100 g. Starch	55.5 g.
100 g. Protein	41.3 g.

Consequently the water deficiency may be partially covered.

Summary.

The influence of various kinds of nutritive food on rats subjected to exsiccose was studied. The results are summarised as follows:—

1. The animals nourished on the diet excess in protein and deficient in the salt mixture suffered loss in body weight most severely when the daily water supply was stopped.

2. Comparing the quantity of water consumed by various diet groups, the one fed on the diet excess in protein was found to take a far greater amount of water than others.

3. The duration of survival after the water was taken from the diet was shortest when the animal was fed on the diet excess in protein and deficient in the salt mixture and longest when fed on the diet excess in fat. The explanation for this was given.

4. When the animals were fed on a diet deficient in the salt mixture they had a tendency to lose water from the tissues faster than when they were fed on any other nourishment.

II. The Metabolic Activity of Tissues in Exsiccose.

Since all of the vital phenomena are displayed in the medium of water, the shortage of water-intake should necessarily result in a serious disturbance in the metabolic activity of the living tissues.

It is interesting, therefore, to study in what manner and to what extent the exsiccose produced experimentally affects the activity of the metabolic ferments. Among them oxidase and reductase seem to have most direct concern with the vital process.

It was, therefore, decided to investigate the behavior of both

ferments under the effect of exsiccose. Further, the content of sulfhydryl compounds, chiefly glutathion was determined, because to this substance many authors attributed a high metabolic importance. The experiment was performed on rat fed on various food stuffs, because as is shown in the foregoing report, different diets have exhibited somewhat different effects when the animal was subjected to exsiccose.

I. The Dehydrogenase Activity in Exsiccose.

The rats were nourished on various diets as described in the previous experiment for about four weeks, water being given ad libitum. Then the water was omitted for the following four days. At the end of this period the rat was killed and the tissues were minced into paste by scissors; 0.1 gm. of this paste was carefully weighed and put into the vacuum tube of Thunberg together with 1.0 cc. of phosphate buffer (pH 7.13) and 1.5 cc. of M/5000 methylene blue solution.

After evacuation had been continued for one and a half minutes at 3 m.m. Hg pressure, the tube was immediately dipped into a thermostat kept at 37°C and the time for complete decoloration was noted. The results are summarised in Table I.

Table I.

Activity of methylene blue reduction of the tissues of the exsiccose rat compared with that of the normal.

Time for complete decoloration (average value of six rats).

	Liver		Heart	
	Normal	Exsiccose	Normal	Exsiccose
Basal Diet.	6′52″	7′35″	13′45″	14′27″
Diet excess in protein.	6′44″	7′15″	14′41″	15′12″
Diet excess in carbohydrate.	8′25″	8′16″	12′46″	13′13″
Diet excess in fat.	14′15″	9′35″	17′30″	16′45″
Diet deficient in salt mixture.	10′05″	9′20″	16′20″	16′35″

From the above table there is no apparent difference in the reducing activity between the exsiccose animal and the normal.

By comparing each diet group it will be seen that the group nourished on a diet excess in fat and deficient in salt mixture showed a slight prolongation of the time of decolorisation.

Since the rate of methylene blue reduction is the function of dehydrogenase activity and the nature and the quantity of H-donator in the tissues, it is important to treat these two factors separately to get a clear insight into the reducing activity of tissues.

Further, according to the recent work of K. Kodama (1932), the active donator found in the living tissue is the compound of the chemical constitution of hexose phosphate. This can be easily extracted from tissue by boiling with diluted alcohol. It was intended, therefore, in the next experiment to determine the content of H-donator in such an alcohol extract of the tissues of the animal fed on different diets as described above. As ferment it is necessary to use a preparation of uniform activity throughout the experiments. For this purpose, beef heart muscle was used. This was washed thoroughly with water until this alone did not reduce methylene blue at all, then minced and ground into paste with sand in a mortar and diluted with water to make a 10% emulsion.

The whole experiment was carried out as follows:—

The fresh tissue to be tested was minced by scissors and made into paste by grinding in a mortar. 2.0 gm. of this paste was carefully weighed out into a small conic flask and mixed with 5 cc. of 70% alcohol. The flask was placed for ten minutes in the thermostat kept at 90°C; then the contents were filtered. 0.5 cc. of the filtrate was introduced into Thunberg's vacuum tube. To this was added 1.0 cc. of M/2500 methylene blue solution, 1.0 cc. of phosphate buffer (pH 7.13) and 1.0 cc. of 10% beef heart emulsion, and the time for decolorization was noted. The results are indicated in Table II.

We can conclude from the above table that the contents of H-donators in the tissue under exsiccose is slightly more abundant than in the normal. Comparing each diet group we found in this case also a result similar to that in the foregoing experiment; namely, the groups fed on diet excess in fat and deficient in the salt mixture showed a slight weakness. It is apparent, therefore,

Table II.
The contents of H-donator in the tissues in exsiccose.

	Liver		Muscle	
	Normal	Exsiccose	Normal	Exsiccose
Basal diet	6'45"	6'05"	9'40"	10'15"
Diet excess in carbohydrates	6'30"	5'25"	8'20"	6'45"
Diet excess in protein	7'26"	6'35"	8'48"	7'40"
Diet excess in fat	8'30"	6'45"	16'45"	14'25"
Diet deficient in salt mixture	9'45"	12'	16'20"	15'48"

that the decrease in the reducing activity in the groups of certain diets can be ascribed to the relative lack of H-donators.

II. The oxidase contents of tissue in exsiccose.

The oxidase here means a ferment, which exerts oxidation of organic molecule by activating molecular oxygen in the sense of Warburg.

The determination of its activity was made by Staemler and Sanders method (1925), modified by K. Shin (1930) in this laboratory. 0.1 gm. of chopped tissue obtained as in the foregoing experiment was carefully weighed out and transferred into a conic flask of 200 cc. capacity, together with 2.0 cc. of phosphate buffer (pH 7.73) and 2.0 cc. of M/150 α-naphthol solution. Then 2.0 cc. of M/150 paraphenylene-diamin solution was added, which contained 10% of M/10 HCl to prevent spontaneous oxidation. After being completely mixed, the flask was put into a thermostat kept at 37.°C. for twenty minutes.

Then the indophenol dye produced was extracted quickly with 10 cc. of xylol and matched colorimetrically against the standard solution of fuchsin. The amount of the dye is indicated conveniently by mg. of fuchsin (Table II).

It is evident from the above results that indophenol synthesis in exsiccose is much less than in the normal. In other words the aerobic oxidation of the tissue in exsiccose is fairly retarded in comparison with the anaerobic.

Table III.
Oxidase contents of tissues in exsiccose compared with normal.

	Liver		Heart	
	Normal	Exsiccose	Normal	Exsiccose
Basal diet	0.051	0.036	0.128	0.098
Diet excess in carbohydrates	0.046	0.032	0.134	0.072
Diet excess in protein	0.062	0.044	0.121	0.077
Diet excess in fat	0.056	0.041	0.116	0.062
Diet deficient in salt mixture	0.057	0.038	0.123	0.063

III. Contents of sulfhydryl compound in exsiccose.

Since the special physiological function of glutathion in the oxidation and reduction processes in living cells has been demonstrated by F. G. Hopkins (1921), the substance has aroused much interest among many investigators.

It is now generally assumed that a close relationship exists between the metabolic activity of a tissue and its contents of sulfhydryl compounds. It was intended, therefore, in the following experiment to determine the contents of sulfhydryl compounds in exsiccose tissues under various conditions of nutrition.

As the method of the determination, Y. Okuda's iodine-method (1925) modified by K. Yamasaki (1931) was applied.

1.0 gm. of leg-muscle, 0.3 gm. of liver, 0.2 gm. of heart were weighed and ground in a mortar with a small portion of pure sea sand. The paste was then mixed thoroughly with 15 cc. of N/2 sulfosalicylic acid and 1 cc. of N. sulfosalicylic acid.

The precipitate produced was filtered off and 5 cc. of the filtrate was put into a titration-vessel of about 50 cc. capacity, and up to 1.0 cc. enough water or N. sulfosalicylic acid was added, taking care to adjust the end concentration of sulfosalicylic acid to N/2. Then each 1.0 cc. of 5% KJ solution and N. sulfosalicylic acid solution were added. The vessel was then put into a small thermostat kept at 20°C. and potentiometrically titrated with

M/2000 KJO_3 solution.

From the quantity of iodate consumed the corresponding amount of cystein was calculated. The cystine content was also measured on the same filtrate after reducing it completely to cysteine, but in my repeated experiments its contents in fresh tissue was very low and often zero both in the case of exsiccose and of normal. The results are indicated in Table IV, where the values pro 1.0 gm. of dry material are given in mg.

Table IV.

Contents of sulfhydryl compounds of tissues in exsiccose under various nutritive conditions.

mg. Cysteine in 1.0 gm dry tissues.

	Liver		Muscle		Heart	
	Normal	Exsiccose	Normal	Exsiccose	Normal	Exsiccose
Basal diet	1.742	1.622	0.223	0.108	0.486	0.378
Diet excess in carbohydrates	1.770	1.231	0.232	0.132	0.502	0.276
Diet excess in protein	1.905	1.335	0.151	0.106	0.533	0.264
Diet excess in fat	1.630	1.341	0.189	0.159	0.401	0.276
Diet deficient in salt mixture	1.692	1.298	0.188	0.147	0.487	0.256

From the above table it is evident that the cysteine content of tissues decreases in exsiccose, especially in muscle and heart. This confirms quite satisfactorily the results of E. Schiff and M. Fukuyama (1928).

Discussion.

From the result of the above experiments we are justified in concluding that in exsiccose the oxidase activity of the tissue shows a marked decrease. This gives an additional evidence to the theory of E. Schiff that in exsiccose a severe disturbance of intermediate metabolism occurs owing to insufficient oxidation. A high grade acidosis, which is often observed in exsiccose, can also be explained in the same way.

One of the cardinal symptoms of infant dyspepsia is a

diarrhea which naturally leads to water deficiency in the body. The assumption therefore may be justified that in addition to the toxic substance produced in the intestine, various factors due to water deficiency depicted in the above experiments may be added to the etiological moments involved in the intoxication.

Further, from the dietetic point of view it may be worth while to mention that in the treatment of dyspepsia an excess of protein in the diet must be avoided, as this causes a higher degree of water deficiency in the body.

III. The Contents of Various Phosphorus Compounds in Tissues in Exsiccose.

W. Straub (1899) was perhaps the first to notice the close relationship between exsiccose and phosphate. In 1899 he observed that in exsiccose there was an increase of phosphate excretion into urine.

E. Schiff and C. Choremis (1926) states that the inorganic phosphate in blood serum increases in the case of hunger when accompanied with water deficiency, while with an abundance of water there is a rather marked decrease. They ascribed this to the brisk breakdown of hexosephosphoricacidester in the exsiccose. But recent advance in muscle physiology has brought a number of phosphoric acid compounds to the front and ascribed to them a significant rôle in the metabolism, especially, of the carbohydrate in the animal. Namely, besides various hexosephosphates described by A. Harden and W. J. Young (1906) C. Neuberg (1918) R. Robinson (1922) and E. Embden, (1924) phosphagen (a compound of creatine and phosphoric acid) and adenylpyrophosphate may be enumerated. The former was discovered by P. Eggleton and G. P. Eggleton (1927) and independently by C. H. Fiske and Y. Subbarow (1929). It is very unstable in acid solution and disappears completely when the muscle enters into a state of rigor. The latter was discovered in muscle by K. Lohmann (1928). This plays a predominant role in the carbohydrate metabolism and fermentation, working as a cozymase.

It seems, therefore extremely interesting and important to investigate the behavior of these compounds in exsiccose with the view of attaining a clearer conception of the pathological aspects of infant toxicosis as well as of obtaining knowledge of the physiological function of those compounds.

The present communication deals chiefly with this problem.

Experiments.

To determine the amount of total phosphate, the true inorganic phosphate, phosphagen, pyrophosphate and hexosephosphate, the method of K. Lohmann (1928) was applied.

Albino rats weighing 30 gm. were nourished on various diets for four weeks, as was noted in the foregoing communication, and then the water supply was completely stopped for the following four days.

Immediately after the rat was killed, 1000-1200 mg. of tissues were weighed out by means of the torsion-balance and placed rapidly in a mortar containing 10. cc. of trichloric acetic acid solution, which was kept under 0°C. by the freezing mixture.

The material was well minced by scissors and thoroughly triturated and then rapidly filtered. 2.0 gm. of the filtrate was pipetted into mixtures of 2.0 cc. Mathison's reagent and 3.0 cc. 1% ammonia solution in a test tube. After twenty-four hours the magnesium-ammonium-phosphate precipitate produced was separated by centrifugalisation and its phosphate content was estimated. This gives the value of true inorganic phosphate.

The above filtrate was kept at room temperature for three hours and then the content of phosphate in 2.0 cc. of the filtrate was estimated. The difference between the above two estimates gives the value of the pohsphagen. Pyrophosphate was determined as follows. 2.0 cc. of the filtrate was mixed with the same amount of 2N. HCl, boiled for just seven minutes and the content of free phosphate was estimated. The value found—(phosphagen + true inorganic phosphate) corresponds to the amount derived from pyrophosphate.

Finally the total phosphor was determined as follows:—

0.2 cc. of 2N. HNO_3 and 1.0 cc. of 2N. H_2SO_4 were added to 2.0 cc. of the same filter. The mixture was heated slowly until it became completely colourless. Then 3.0 cc. of water was added and the whole was heated again in order to convert metaphosphoric acid into orthophosphoric acid and its phosphate content was determined, which represents the total acid soluble phosphoric acid.

From this value the phosphate corresponds to hexosephosphate and adenylic acid was calculated as follows:—

[Total phosphoric acid—(true inorganic phosphate + phosphagen phosphate + pyrophosphate)]

In the following table the average value of six determinations were given. Each value represents the amount in mg. P pro 1.0 gm. tissue of dry weight.

Tabelle I.

Amount of various phosphorus compounds of liver in exsiccose under various nutritive conditions.

mg. P in 1.0 gm. Dry Liver.

		Body Wgt.	True inorganic Phos.	Phosphagen	Pyrophosphate	Hexose-P Adenylic Acid	Total Acid Soluble. P.
Basal Diet	Normal	92 gm	1.180	0.135	0.309	0.092	1.716
	Exsicc.	80 „	1.448	0.103	0.191	0.031	1.776
Diet Excess in Carbohydrates.	Normal	83 „	1.177	0.035	0.294	0.076	1.612
	Exsicc.	83 „	1.107	0.125	0.130	0.038	1.400
Diet Excess in Protein	Normal	78 „	1.374	0.174	0.259	0.094	1.901
	Exsicc.	52 „	1.454	0.110	0.158	0.038	1.760
Diet Excess in Fat	Normal	56 „	1.280	0.072	0.335	0.094	1.759
	Exsicc.	55 „	1.460	0.130	0.095	0.050	1.745
Diet Deficient in Salt Mixture	Normal	73 „	1.106	0.112	0.345	0.074	1.638
	Exsicc.	52 „	1.306	0.098	0.167	0.039	1.610

Tabelle II.

Amounts of various phosphorus compounds of rat muscle in exsiccose under various nutritive conditions.

mg. P in 1.0 gm. Dry Muscle.

		Body Wgt.	True inorganic Phos.	Phosphagen	Pyrophosphate	Hexose-P Adenylic Acid	Total Acid Soluble P.
Basal Diet	Normal	92 gm	2.441	0.720	1.672	0.488	5.321
	Exsicc.	80 „	2.804	0.783	1.441	0.185	5.216
Diet Excess in Carbohydrates.	Normal	83 „	2.425	0.798	1.300	0.300	4,823
	Exsicc.	83 „	2.633	0.939	1.283	0.075	4.933
Diet Excess in Protein	Normal	78 „	2.441	0.711	1.538	0.345	5.035
	Exsicc.	52 „	3.373	0.970	0.893	0.214	5.456
Diet Excess in Fat	Normal	56 „	3.235	0.379	1.661	0.534	5.809
	Exsicc.	55 „	3.195	0.592	0.813	0.230	4.830
Diet Deficient in Salt Mixture	Normal	73 „	2.332	0.754	1.609	0.429	5.124
	Exsicc.	52 „	2.491	0.950	1.122	0.118	4.681

From the above experiments it is clear that the contents of pyrophosphate and of hexosephosphate fraction showed a marked decrease in exsiccose, while those of phosphagen and inorganic phosphate showed no appreciable change. Basing on the postalution of K. Lohmann (1931) on the significance of pyrophosphate, we are lead to conclude that in exsiccose the carbohydrate metabolism is retarded to a certain extent. The fact that hexosephosphate is diminished also stands in accord with this conclusion.

Between each diet group no apparent difference was observed.

Summary.

The amounts of various phosphorus compounds in tissues, namely phosphagen, true inorganic phosphate, pyrophosphate, the sum of hexosephosphate and adenylic acid were determined in

exsiccose and compared with those of the normal.

The results are summarised as follows:—

1. The pyrophosphate and hexosephosphate fraction showed a marked decrease, while phosphagen, true inorganic phosphate showed no such change.

2. Various sorts of nutrition have brought about no appreciable change in the distribution of various phosphoric acid compounds.

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STUDIES ON THE CHANGE IN THE AMOUNT OF CHOLESTEROL AND PHOSPHORUS COMPOUNDS OF CANCEROUS TISSUE AT VARIOUS PERIODS OF ITS GROWTH.

By

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I. Introduction.

In 1924 Dr. Iwano published from this laboratory a paper concerning the chemical composition of cancerous tissue obtained by inoculation of Flexner-Jobling rat carcinoma. He confirmed in that paper the findings of Bennett (1914) that the cholesterol content of cancerous tissue increases with the age of the tumor. Iwano found further that lecithine content remains almost the same throughout the entire tumor life. He also estimated in some of his cases the amount of cholesterol and Lecithine in its peripheral and central parts separately, and found the content of cholesterol in the central portion of cancerous tissue much higher than in the peripheral portion. He performed this separate determination, however, only at the stage of 52nd day after the inoculation, and it seems to me very desirous to follow the change at several stages of cancer growth in both central and peripheral portion of tumor separately. To get a more thorough knowledge of chemical change during the growth of the tumor it is necessary to make first a comparative study among different constituents of tumor tissue following each step of its growth. As this kind of research has been quite meagre, I have made some analytical studies on the contents of several constituents of cancerous tissue including nitrogen, inorganic P, acid soluble organic P, lipid P, protein P, free cholesterol and ester cholesterol, the result of which is given in the following sections.

II. Material and Methods.

1. *Cancerous tissue:* Scissored particles of Flexner-Jobling rat carcinoma, the strain obtained from the pathological Institute of Jikeikwai Medical School, was washed well with physiological salt solution and inoculated in an amount of 0.4 cc. into the subcutaneous tissue on the back of an albino rat weighing about 60 gm. All the animals were fed on wheat and green vegetables. Free access to water was also provided.

The takes of implanted tumor amounted to about 80 per cent, except in the middle of the summer or winter when the percentages are quite low. The size of the tumor was about 1.6 cm. long and 1 cm. wide at the end of two weeks, 2.5 cm. and 2 cm. resp. at the end of three weeks, and the size increased to about 3.5 and 2.5 cm. respectively on the 30th day. About this time the presence of a cyst became palpable, which increased in size or burst on about the 40th day after inoculation or was stopped in growth by the death of the animal, although in some rare cases the cyst could be reabsorbed. When the cyst bursts the life of the animal is rather prolonged. At about the end of six weeks, the size of the tumor may equal the entire body of the animal.

At several stages of tumor growth 5–13 samples of equal sized tumors were selected from animals in the same period after the inoculation.

2. *Tumor paste:* The needed number of tumors were obtained by enucleation and cautiously freed from other tissues. The tumor tissue was then separated with scissors into central and peripheral portions, and separately made with a pistil and mortar into a homogeneous paste. An aliquote portion of this paste was used for the analysis. The cyst content was analysed separately.

3. *Content of water:* About 1 gm. of tumor paste was weighed in a weighing bottle; about three times its volume of alcohol was added, and the mixture was stirred well and evaporated on the water bath. The addition of alcohol and the evaporation was repeated twice more and dried to weight constant at 105°C. The

content of water and dry material were calculated in a percentage value.

4. *Total nitrogen:* ca. 0.5 gm. of tissue paste was oxidised according to Kjehldahl and the oxidised mixture was filled to 50 cc., 5 cc. of which was used for alcaline distillation and titration of ammonia. The content of nitrogen was expressed in the percentage value of both wet and dry materials.

5. *Inorganic phosphorus:* 1–1.5 gm. of tissue paste were treated in the mortar with 2 cc. of 1% HCl to destroy the enzymic action and well grinded with emery grain. 5 cc. of 25% trichloracetic acid and 20 cc. of distilled water were then added, well stirred and filtered after one hour. 1–3 cc. of filtrate were used for phosphorus determination by Naito's method (1928).

6. *Acid soluble total phosphorus:* 1–3 cc. of the filtrate used for the determination of inorganic phosphorus were oxidised with addition of 3 cc. of 20% H_2SO_4 and 1 cc. of concentrated HNO_3, nitrosylsulphuric acid was destroyed with addition of water, and nitric acid was freed by distillation. The total amount of the remaining mixture was used for phosphorus determination by Naito's method.

7. *Acid soluble organic phosphorus:* The difference between the amount of acid soluble total phosphorus and that of inorganic phosphorus was taken as the content of acid soluble organic phosphorus.

8. *Lipid phosphorus:* 1–1.5 gm. of tissue tumor paste were mixed with 2–3 times its volume of alcohol, evaporated on the water bath and dried in the drying oven. The residue was extracted with ca 50 cc. of alcohol by the use of Kumagawa–Suto's alcohol extractor for about 7 hours. The alcoholic extract was then evaporated and well dried. The residue was then taken up with ether. Etherial extract was evaporated and dissolved into 50–100 cc. of alcohol. 5 cc. of this alcoholic solution were evaporated and digested upon the addition of 3 cc. of 20% sulphuric acid and 1 cc. of concentrated nitric acid. Phosphoric acid was then determined by Naito's method.

9. *Total phosphorus:* ca. 0.5 gm. of cancer paste were des-

troyed in a digestion tube with the addition of 5 cc. of 20% H_2SO_4 and 1 cc. of concentrated nitric acid, and the resulting mixture was diluted to 50 cc. in a measuring flask. 3–5 cc. of this solution were used for phosphorus determination.

10. *Organic total phosphorus:* The organic total phosphorus was calculated by the difference between total phosphorus and inorganic phosphorus.

11. *Protein phosphorus:* Protein phosphorus was obtained by the subtraction of the sum of acid soluble total phosphorus and lipid phosphorus from the total phosphorus.

12. *Free cholesterol:* Cholesterol was extracted from 2–5 gm. of cancer paste by Onizawa's method (1928). 5–20 cc. to 100 cc. chloroform extract were used for free cholesterol by Onizawa's method.

13. *Total cholesterol:* 5–20 cc. of chloroform solution of extraction cholesterol described under section 12 were put into 100 cc. Erlenmeyer flask, evaporated on the steam-bath and saponified with the addition of 7–10 cc. of saturated alcoholic caustic soda solution by continuous heating for 6 hours under reflux condensor. The extraction and determination of cholesterol remained just the same as described above.

14. *Ester cholesterol:* Ester cholesterol was calculated by subtracting this amount of free cholesterol from the total cholesterol.

III. Result.

The contents of several different constituents of cancerous tissue determined at several periods are given in tables I, II and figures 1, and 2.

The amounts of several different constituents other than water content are given in the percentage value of dry material.

From the tables and figures the following points may be pointed out.

1. *Water content:* The content of water is a little higher in the central portion than in the peripheral portion. It increases also with the age of the tumor.

TABLE I.

The contents of several constituents of cancerous tissue at different stages of its growth.

Days after inoculation	number of analysis	portion	solid substance %	water content %	Phosphorus				cholesterol		Total N %
					acid soluble		lipid %	total %	free %	total %	
					inorganic %	total %					
14	I	peripheral	20.2	79.8	0.362	0.510	0.278	1.46	0.906	1.19	11.8
		central	19.0	81.0	0.705	0.874	0.320	1.59	1.11	1.53	12.4
	II	peripheral	19.3	86.7	0.310	0.554	0.294	1.45	0.927	1.25	11.9
		central	18.1	81.9	0.520	0.740	0.376	1.70	1.20	1.56	12.8
	III	peripheral	21.9	78.1	0.310	0.553	0.321	1.54	—	—	12.5
		central	21.0	79.0	0.453	0.676	0.309	1.67	—	—	12.2
21	I	peripheral	19.1	80.9	0.473	0.576	0.248	1.43	1.16	1.59	12.0
		central	18.5	81.5	0.735	0.810	0.254	1.55	1.54	2.02	11.9
	II	peripheral	19.3	80.7	0.452	0.575	0.251	1.46	1.31	1.62	11.8
		central	18.1	81.9	0.707	0.785	0.278	1.64	1.50	2.01	11.8

TABLE I (continued)

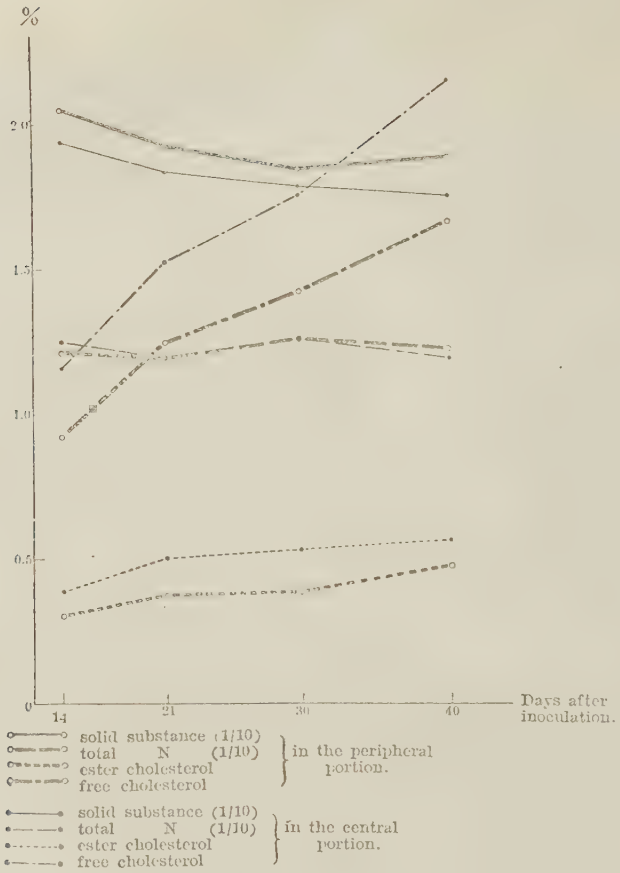
Days after inoculation	number of analysis	portion	solid substance %	water content %	Phosphorus				cholesterol		Total N %
					acid soluble		lipid %	total %	free %	total %	
					inorganic %	total %					
30	I	peripheral	18.0	82.0	0.548	0.600	0.234	1.56	1.38	1.76	12.6
		central	17.4	82.6	0.816	0.897	0.224	1.67	1.71	2.21	12.5
	II	peripheral	18.7	81.3	0.583	0.652	0.224	1.56	1.44	1.82	12.3
		central	18.0	82.0	0.861	0.944	0.218	1.65	1.76	2.32	12.4
40	I	peripheral	18.9	81.1	0.640	0.720	0.194	1.49	1.63	2.12	12.4
		central	17.6	82.4	1.09	1.16	0.201	1.66	2.14	2.79	12.1
		cyst	8.3	91.7	0.264	0.357	0.072	0.536	0.766	0.931	12.5
	II	peripheral	18.4	81.6	0.668	0.690	0.216	1.60	1.65	2.09	11.8
		central	17.0	83.0	1.06	1.11	0.188	1.67	2.09	2.57	11.5
		cyst	6.3	93.7	0.473	0.484	0.132	0.654	0.590	0.897	10.7

TABLE II.

The average contents of several constituents of cancerous tissues at different stages of its growth.

Days after inoculation	portion	solid substance %	water content %	Phosphorus							cholesterol			Total N %
				acid soluble			lipid %	protein %	organic %	total %	free %	ester %	total %	
				inorganic %	organic %	total %								
14	peripheral	20.5	79.5	0.327	0.212	0.539	0.298	0.64	1.15	1.48	0.917	0.30	1.22	12.1
	central	19.4	80.6	0.559	0.204	0.763	0.335	0.55	1.09	1.65	1.16	0.39	1.55	12.5
21	peripheral	19.2	80.8	0.463	0.113	0.576	0.250	0.62	0.99	1.45	1.24	0.37	1.61	11.9
	central	18.3	81.7	0.721	0.077	0.798	0.266	0.54	0.88	1.60	1.52	0.50	2.02	11.9
30	peripheral	18.3	81.7	0.566	0.060	0.626	0.229	0.71	0.99	1.56	1.41	0.38	1.79	12.5
	central	17.7	82.3	0.839	0.082	0.921	0.221	0.52	0.82	1.66	1.74	0.53	2.27	12.5
40	peripheral	18.6	81.4	0.654	0.051	0.705	0.205	0.64	0.90	1.55	1.64	0.74	2.11	12.1
	central	17.3	82.7	1.07	0.07	1.14	0.195	0.34	0.60	1.67	2.12	0.56	2.68	11.8
	cyst	7.3	92.7	0.369	0.052	0.421	0.102	0.072	0.227	0.595	0.678	0.236	0.914	11.6

Fig. 1

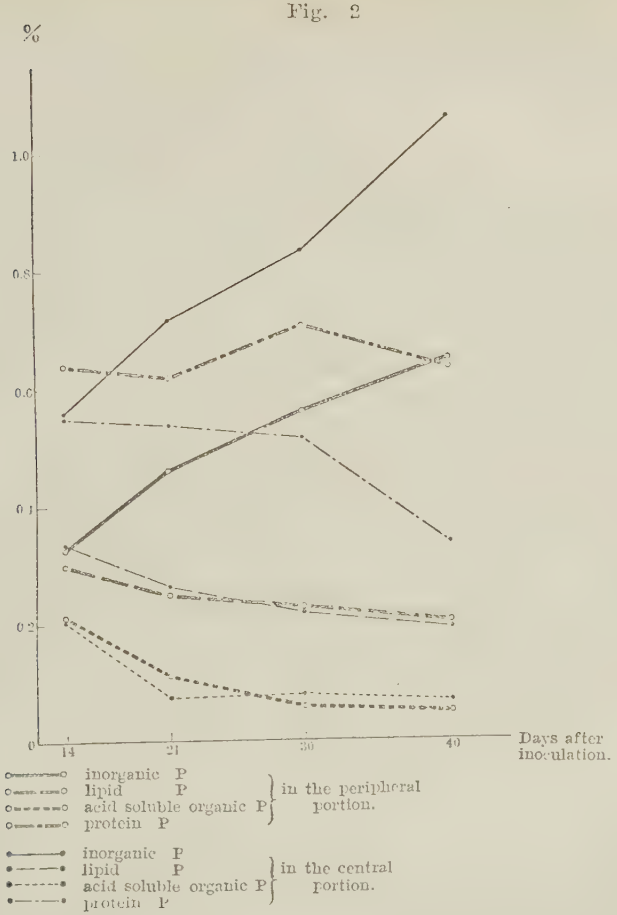


2. *Nitrogen content:* The amount of total nitrogen does not differ much between central and peripheral portions. It remains also almost the same through all stages of the tumor.

3. *Cholesterol:* The central portion of the tumor is always rich in both free and combined cholesterol. The free cholesterol increases steadily with the age of tumor. Its increase in the central portion at the later period is especially noticeable.

4. *Total phosphorus:* There is always more total phosphorus in the central portion of tumor than in its peripheral portion

Fig. 2



through all stages of tumor growth. Variation with regards to the age of tumor can not be observed clearly.

5. *Inorganic phosphorus:* The central portion contains about 70% more of inorganic phosphorus than the peripheral portion. With the age of tumor a considerable increase of inorganic phosphorus is observed, both in the central and in the peripheral portion and in an about the same degree.

6. *Acid soluble organic phosphorus:* The content of acid soluble organic phosphorus does not differ much between the cen-

tral and peripheral portion and tends to decrease with the age of the tumor. The decrease of acid soluble organic phosphorus in the peripheral portion is, however, a little larger than that in the central portion.

7. *Lipid phosphorus:* The amount of lipid phosphorus is almost the same in the central and peripheral portions and shows nearly the same rate of decrease with the age of tumor life.

8. *Protein phosphorus:* The peripheral portion of cancerous tissue contains more of protein phosphorus than the central portion. The protein phosphorous decreases with the age of the tumor, especially after 30 days of inoculation, while in the peripheral portion it remains almost unchanged.

IV. Discussion.

If we look at the tables and figures it may be perceived clearly that the constituents of tumor tissue enumerated above can be divided into three groups according to their increase or decrease during tumor growth.

Constituents showing neither increase nor decrease.

1. *Nitrogen content* always remains constant both in the central and peripheral portions, although, as will be considered below, the amount of protein, judged from the content of protein phosphorus, is decreased in the central portion at the sixth week after inoculation. This shows that the decomposition product of protein is retained largely in tumor tissue. We did not, however, study this point in the distribution of nitrogenous matter.

The content of protein in the peripheral portion also remains constant through all stages of tumor growth. This is rather natural as in this region the tumor cells are yet vigorous enough and retain their structure wholely.

2. *Total phosphorus* only shows quite a little variation through all stages of the tumor growth although the amounts of their individual constituents change with the age of tumor. The

content of total phosphorus seems to be especially constant in the central portion.

Constituents which increase during the tumor growth.

As stated just above we did not study the change in nitrogenous matter but limited our consideration mainly to the change of phosphorus compounds and cholesterol. Among phosphorus compounds inorganic phosphorus is the only compound which increases with the life of the tumor.

1. *Inorganic phosphorus:* Inorganic phosphorus increases steadily with the age of the tumor both in the central and peripheral portions. This increase is counteracted largely by the decrease of acid soluble organic phosphorus and lipid phosphorus in the peripheral portion, while in the central portion the destruction of protein plays an important role. The degree of increase of inorganic phosphorus amounts to 90–100%.

2. *Cholesterol:* Both free and combined form of cholesterol increases with the growth of the tumor. While at the end of two weeks the amounts of free cholesterol counted 1.16% in the central portion and 0.92% in the peripheral portion, they increased on the fortieth day after inoculation up to 2.11% and 1.64% respectively. The increase counted about 80%. As the table and figure show, the increase in ester cholesterol was also obvious, amounted to 57 in the peripheral portion and 43% in the central, although the absolute amounts of increased ester cholesterol are far below those of free cholesterol.

The constituents which decrease during the tumor growth.

1. *Protein phosphorus in the central portion decreases with the progress of tumor growth.* The decrease is especially noticeable at the sixth week, when the cyst promptly enlarges. This decomposition of phosphorus containing cell protein seems to be the main cause of a large increase of inorganic phosphorus in the

central portion at this stage of tumor life.

2. *Acid soluble organic phosphorus:* The amount of acid soluble organic phosphorus decreases in both portions of the tumor during the course of its growth. The degree of decrease is, however, much larger in the peripheral portion than in the central portion. The amount of acid soluble organic phosphorus in the peripheral portion decreases on 40th day to 24% of that on 14th day, while in the central portion the corresponding value is about 34%.

3. *Lipid phosphorus:* Lipid phosphorus shows a steady decrease with the tumor growth both in the central and peripheral portions. The degree of decrease amounts to 42% in the central portion and 31% in the peripheral portion.

These results indicate clearly the fact that in the course of tumor growth there occurs a decrease of lipid phosphorus and of acid soluble organic phosphorus with a corresponding increase in inorganic phosphorus on the one hand, while on the other there appears a noticeable increase of cholesterol, especially in its free form. The sudden decrease of protein phosphorus in the central part with the simultaneous sudden enlargement of cyst at the latest stage naturally shows us the increasing decomposition of cell material at this period. The curves of decreasing lipid phosphorus and acid soluble organic phosphorus and those of increasing cholesterol suggest to us that an incessant decrease in cell activity is occurring during the progress of tumor growth. To follow this change of cell activity we determined the activity of hexosemonophosphatase at several stages of tumor growth. The hexosemonophosphate was prepared from rabbit muscles, following the method of Lohmann (1928). 0.5 gm. of tissue paste was added with 10 cc. of Borax-KCl-NaOH buffer solution (PH 9.0) and 10 cc. of 0.13% sodium hexosemonophosphate and incubated at 37° for 3 hours. Then 5 cc. of 25% trichloracetic acid solution were added, filtered and an aliquote portion of the filtrate was used for the determination of inorganic phosphorus. The amount of decomposed hexosemonophosphate phosphorus is calculated by subtracting from the amount of this phosphorus that in the control experi-

ment, where water was used instead of hexosemonophosphate solution.

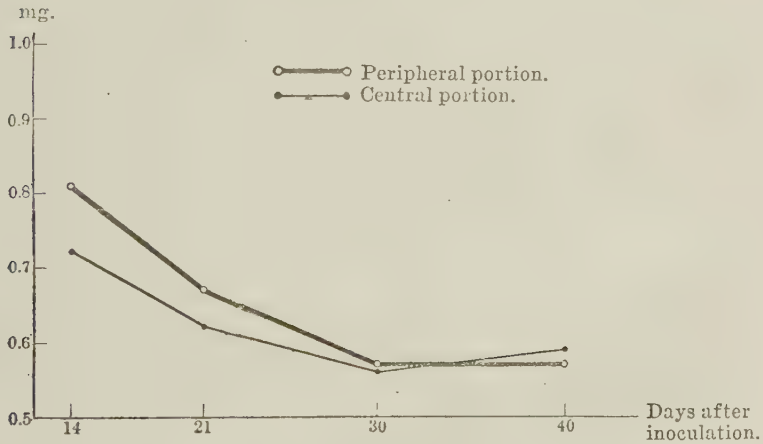
The result is shown in table III, and figure 3.

TABLE III.

The decomposition of hexosemonophosphate by the cancerous tissues at different stages of its growth.

	P in mg. splitted by 1 gm. of cancer paste			
Days after inoculation	14	21	30	40
peripheral portion	0.81	0.67	0.57	0.57
central portion	0.72	0.62	0.56	0.59

Fig. 3



As can be seen from the table and figure, the activity of hexosemonophosphatase is decreasing with the tumor growth. Although we did not measure the change of amount of oxygen consumption of tumor tissue in the course of its growth owing to the lack of time and so far as I know there has been no work ever done on this point, it is quite probable that the oxygen consumption will also decrease with the course of tumor growth.

V. Summary.

1. The amount of water, nitrogen, cholesterol bodies and different kinds of phosphorus compounds were determined at several stages of carcinoma growth.

2. The water content of the tumor tissue increases a little with the age of the tumor.

3. The content of nitrogen remains almost constant during the entire tumor growth.

4. Protein is only decomposed in the latest stage and only at the central portion.

5. Cholesterol, both free and combined, increases steadily with the growth of the tumor.

6. The amount of lipid phosphorus and acid soluble organic phosphorus steadily decreases during the growth of the tumor.

7. Together with the decrease of lipid phosphorus, acid soluble organic phosphorus and protein phosphorus, the inorganic phosphorus promptly increases through the whole life of the tumor.

8. The activity of phosphatase tends to decrease with the tumor growth.

I wish to express my sincere thanks to Prof. S. Kakiuchi for his kind advice throughout the course of this investigation.

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ÜBER DEN EINFLUSS DER GALLENSÄURE AUF GLYCEROPHOSPHATASE (III).

Von

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In der ersten Mitteilung (1931) wurde bereits berichtet, dass die Wirkung der Glycerophosphatase in der Niere und Leber in vitro durch Zusatz von Cholsäure deutlich gehemmt wird. Bekanntlich sind Niere und Leber Ausscheidungsorgane für die Phosphorsäure. Durch die Untersuchungen von Kawada (1931), Hatakeyama (1927), Sekitoo (1929) und Fuziwara (1931) wurde bereits bewiesen, dass bei Zufuhr von Gallensäure oder bei experimentellem Stauungsikterus im Kaninchenorganismus die Phosphorsäureausscheidung im Harn, ebenso wie die in der Galle des Fistelhundes, durch orale Zufuhr der Gallensäure vermehrt wird.

Diese Autoren haben dabei betont, dass die vermehrte Phosphorsäureausscheidung im Harn und in der Galle auf der durch die Wirkung der Gallensäure herbeigeführten Steigerung des Nucleinstoffwechsels beruht. In diesem Sinne ist es von Bedeutung, die Wirkung der Gallensäure auf Knochenphosphatase zu erforschen, um das Wesen der bei Zufuhr der Gallensäure vermehrten Phosphorsäure im Harn und in der Galle klarzustellen, weil als Quelle der Phosphorsäure im Harn und in der Galle ausser dem Nuclein anorganische Phosphorsäure und Phosphatide in Betracht kommen.

Was die Phosphatase des Knochens anbetrifft, so wurde von Robison (1923) bereits nachgewiesen, dass der Knochenextrakt den Hexosephosphorsäureester, dessen Phosphorsäure nach Robison für die Knochenbildung zur Verfügung gestellt wird, und den Glycerinphosphorsäureester spaltet, und dass bei Rachitis diese

Knochenphosphatase sich vermehrt. Nach Takahashi (1924) und Fujiwara u. Ito (1925) soll die Hexosemonophosphorsäure im Sinne von Robison durch Femurextrakt gespalten werden. Auch hat Demuth (1925) beobachtet, dass der Extrakt des rachitischen Knochens und Knorpels den Hexosediphosphorsäureester wesentlich stärker spaltet als der Extrakt von nicht rachitischen Knochen. Natürlich ist der Gehalt des Knochens an Phosphatase je nach dem Alter des Tieres ganz verschieden, und immer je grösser, je jünger das Tier ist. Nach Kay u. Robison (1924) sind 2 Phosphorsäureester im Blut vorhanden, und der eine wird durch den Knochenextrakt leicht gespalten. Aber Robison u. Soames (1924/25) haben in ihrem Experiment betont, dass die mangelhafte Knochenverkalkung bei Rachitis weder auf dem Phosphatasegehalt im Knochen noch auf der Phosphorsäure im Blut beruht.

Es ist jedoch bemerkenswert, dass bei Tieren mit einer Diät, die sehr arm an anorganischem Phosphor und fettlöslichem Vitamin D ist, die Konzentration des anorganischen Phosphors und des Phosphorsäureesters beträchtlich unter normal sinkt, und dass die Konzentration des anorganischen Phosphors durch Hinzufügen von Lebertran, in welchem Vitamin D enthalten ist, fast wieder zur Norm zurückgeführt wird. Nach Robison und seinem Mitarbeiter soll sich das Calciumphosphat im Knorpel anlagern, wenn der rachitische Knochen mit der Lösung von Calcium-Monophosphat oder Calcium-Glycerophosphat in Berührung kommt, und die Knochenphosphatase mit der Knochenbildung in innigem Zusammenhang stehen.

Korenschevsky u. Carr (1925), Pfannenstiel (1927), Kreitmair u. Hintzelmann (1928), Baumgartner, King u. Page (1929) und Page u. Reside (1930) haben in ihren mühevollen Untersuchungen bewiesen, dass der Calciumstoffwechsel im Knochen mit dem Gehalt der Nahrung an Vitamin D und mit dem Knochenenzym in innigem Zusammenhang steht, indem die Phosphatase des Knochens bei der Mobilisation und Anlagerung des Calciumphosphates eine grosse Rolle spielt.

Was den Einfluss der Hormone auf den Phosphatasegehalt im

Knochen anbetrifft, so wurde von vielen Autoren, wie Heymann (1930), Page (1930) gefunden, dass die Wirkung der Knochenphosphatase durch Parathhormon sowohl in vitro als auch in vivo gehemmt wird. Aus den Ergebnissen haben sie die Ansicht gefolgert, dass die Mobilisierung des Calciums und der Phosphorsäure mit der Enzymwirkung der Knochen eng verknüpft ist.

Im hiesigen Institut hat Kimura (1931) beobachtet, dass sowohl die anorganische als auch die organische Phosphorsäure im Blut durch Zufuhr von Gallensäure vermehrt wird. Nach Sekitoo (1930) wird bei Gallensäureverlust im Kaninchenorganismus eine Hypocalcämie, bei überschüssiger Zufuhr von Gallensäure dagegen Hypercalcämie erzeugt. Daraus hat er geschlossen, dass die Gallensäure zur Regulation des Calciumstoffwechsels in inniger Beziehung steht. Aus allen oben angeführten Gründen ist es sehr interessant, die Wirkung der Gallensäure auf Knochenphosphatase im Sinne eines Hormons zu erforschen.

Experimenteller Teil.

1. Einfluss der Gallensäure auf Glycerophosphatase im Knochen.

Die Fermentlösung wurde in der Weise bereitet, dass der von Fleisch und Blut sorgfältig befreite Extremitätenknochen des Kaninchens in einer Reibschale mit der zehnfachen Gewichtsmenge Chloroformwasser gründlich zerrieben und 2 Tage lang im Brutschrank bei 37°C. der Autolyse unterworfen wurde, worauf das Autolysat 2 Tage lang in fliessendem Wasser dialysiert wurde. Dieses Dialysat wurde als Fermentlösung zum Versuch verwendet. Als Substrat wurde β-Glycerinphosphorsaures Natrium (E. Merck) verwendet. Die fermentative Hydrolyse wurde im Brutschrank bei 37°C. unter Pufferung mit Glykokoll und Natronlauge ausgeführt. Nach 24stündiger Hydrolyse wurde die Versuchslösung mit 10 ccm einer 5%igen Trichloressigsäure versetzt, von der Fällung abfiltriert, und dann die frei gewordene Phosphorsäure bestimmt. Die Versuchsanordnungen sind in den folgenden Tabellen I u. II zusammen angegeben. Die angegebenen Zahlen

der Tabellen III u. IV zeigen den mg %igen Phosphorgehalt. Die Pufferlösung hat $P_H = 8{,}9$.

Tabelle I. (Versuchsanordnung.)

	A	B	C	D
1% Natr. Cholat (ccm)	0	1	3	5
1% Natr. Glycerophosphat (ccm)	10	10	10	10
Pufferlösung (ccm)	10	10	10	10
Fermentlösung (ccm)	10	10	10	10
Wasser (ccm)	10	9	7	5
Toluol (ccm)	1	1	1	1

Tabelle II. (Versuchsanordnung.)

	A	B	C
0,25% Natr. Cholat (cc)	0	1	2
1% Natr. Glycerophosphat (ccm)	10	10	10
Pufferlösung (ccm)	10	10	10
Fermentlösung (ccm)	10	10	10
Wasser (ccm)	10	9	8
Toluol (ccm)	1	1	1

Tabelle III. (P. in mg%)

Nr. d. Versuchs	A	B	C	D
1	19,53	16,29	14,53	12,81
2	19,53	15,23	13,78	12,67
3	19,11	15,72	13,50	12,49
4	19,39	15,51	13,50	12,60
5	19,52	15,79	13,85	12,69
6	19,73	16,75	15,02	13,85
7	19,73	17,17	15,56	12,50
Durchschnittswert	19,51	16,07	14,25	12,80
Befreite P. (%)		82,4	73,0	65,6
Cholatgehalt (%)	0	0,025	0,075	0,125

Tabelle IV. (P. in mg%)

Nr. d. Versuchs	A	B	C
1	16,04	15,69	15,51
2	16,04	15,73	15,07
3	15,96	15,33	15,16
4	15,60	15,29	14,16
5	15,69	15,29	14,71
6	15,96	15,25	14,94
7	15,82	15,51	15,11
Durchschnittswert	15,87	15,43	14,95
Befreite P. (%)		97,2	94,2
Cholatgehalt (%)	0	0,00625	0,0125

Aus den Tabellen III u. IV erhellt, dass die Abspaltung der Phosphorsäure aus der Glycerinphosphorsäure durch Knochenphosphatase durch Zusatz von Cholsäure im allgemeinen herabgesetzt wird, obwohl diese Herabsetzung je nach dem Gehalt an Cholsäure in der Versuchslösung ganz verschieden ist. Es wird nämlich bei 0,00625%igem Gehalt an Cholsäure die Phosphorsäurespaltung im Vergleich mit der der Kontrolle durchschnittlich 2,8% bei 0,0125%igem Gehalt 5,8%, bei 0,025%igem Gehalt 17,6%, bei 0,075%igem Gehalt 27,0% und bei 0,125%igem Gehalt 34,4% herabgesetzt. Somit wird die Phosphorsäureabspaltung aus Glycerinphosphorsäure durch Knochenphosphatase durch die Wirkung der Cholsäure je stärker gehemmt, je mehr Cholsäure die Versuchslösung enthält.

2. *Einfluss der Gallensäure auf Hexosemonophosphatase im Knochen.*

Die Fermentlösung wurde in genau gleicher Weise bereitet, wie vorher angegeben, und zwar wurde zur Digestion des Knochenbreis eine 20fache Gewichtsmenge von Chloroformwasser gebraucht, und das Gemisch 3 Stunden lang im Brutschrank bei 37°C. digeriert. Die mehrmals filtrierte Lösung wurde als Fermentlösung

in den unten angegebenen Mengenverhältnissen (siehe Tabelle V) zum Versuch verwendet. Als Substrat wurde Fruktosemonophosphorsaures Natrium gebraucht, welches aus Fruktosemonophosphorsaurem Calcium (Yatoconin: Shionogi) nach Meyerhof (1918) durch Versetzung mit Natriumoxalat erhalten wurde.

Tabelle V. (Versuchsanordnung.)

	A	B	C	D	E	F	G
0,5% Hexosemonophosphorsaures Natr. (ccm)	10	10	10	10	10	10	0
0,5% Natr. Cholat (ccm)	0	0,3	0,5	1	2	0	0
Wasser (ccm)	5	4,7	4,5	4	3	5	10
Pufferlösung (ccm)	5	5	5	5	5	5	5
5% Knochenextrakt (ccm)	5	5	5	5	5	0	5
Toluol (ccm)	0,5	0,5	0,5	0,5	0,5	0,5	0,5

10 ccm der frisch bereiteten 0.5% Natrium-Hexosemonophosphatlösung ergeben durchschnittlich 4,70 mg anorganischen Phosphor. Die Pufferlösung besteht aus m/10 Glykokoll und m/10 Natronlauge und hat $P_H = 8{,}9$.

Tabelle VI. (P. in mg.)

Nr. d. Versuchs	A	B	C	D	E	F	G
1	1,95	1,95	1,93	1,91	1,89	0,23	0,14
2	1,96	1,93	1,91	1,89	1,89	0,26	0,10
3	1,89	1,84	1,83	1,83	1,81	0,29	0,14
4	1,95	1,84	1,81	1,79	1,79	0,26	0,14
5	1,96	1,95	1,86	1,83	1,76	0,29	0,16
6	1,91	1,88	1,84	1,84	1,81	0,26	0,16
7	1,95	1,86	1,84	1,84	1,83	0,23	0,14
Durchschnittswert	1,94	1,88	1,86	1,84	1.82	0,26	0,14
Abgespaltene P. (mg)	1,54	1,48	1,46	1,44	1,42		
Abgespaltene P. (%)	32,5	31,2	30.8	30,4	29,9		
Cholatgehalt (%)	0	0,006	0,01	0,02	0,04		

Aus Tabelle VI ist ersichtlich, dass die Spaltung der Phosphorsäure aus der Hexosemonophosphorsäure durch Hexosemonophosphatase im Knochen durch Zufuhr von Cholsäure im allgemeinen herabgesetzt wird, obwohl diese Herabsetzung je nach dem Gehalt an Cholsäure ganz verschieden ist. Die abgespaltene Phosphorsäuremenge aus Hexosemonophosphorsäure durch Knochenphosphatase beträgt beim Kontrollversuch durchschnittlich 32,5%, bei 0,006%igem Gehalt an Cholsäure 31,2%, bei 0,01%igem 30,8%, bei 0,02%igem 30,4% und bei 0,04%igem 29,9%. Genau wie die Glycerophosphatase in der Niere und Leber wird auch hier die Phosphorsäurespaltung aus Hexosemonophosphorsäure je stärker durch die Cholsäure gehemmt, je mehr Cholsäure in der Versuchslösung enthalten ist.

3. Einfluss der Gallensäure auf den Gehalt an Knochenphosphatase bei Meerschweinchen.

Zum Versuch wurden junge Meerschweinchen verwendet, denen täglich einmal 0,3 ccm einer 1%igen Natrium-Cholatlösung pro 100 g Körpergewicht fünf Tage lang subcutan injiziert wurde. 5 Stunden nach der letzten Injektion wurden die Tiere getötet. Der Knochenextrakt wurde in der Weise bereitet, dass eine bestimmte Menge von Extremitätenknochen, von Fleisch und Blut sorgfältig befreit, in einer Reibschale gründlich nach Takahashi (l. c.) verrieben und unter Zusatz einer 20 fachen Gewichtsmenge Chloroformwasser 3 Stunden lang im Brutschrank bei 37°C. digeriert wurde. Das Digestionsgemisch wurde nunmehr filtriert, und das Filtrat in dem unten (Tabelle VII) angegebenen Mengenverhältnissen zum Spaltungsversuch benutzt. Als Substrat wurde Natrium-Glycerophosphat (E. Merck) gebraucht, welches 11,37 %igen Phosphor enthält. Im Präparate wurde keine freie Phosphorsäure nachgewiesen. Als Kontrolle wurde eine Reihe von normalen jungen Meerschweinchen zur Bestimmung des Phosphatasegehalts des Knochens getötet und in genau gleicher Weise behandelt. Als Massstab für den Phosphatasegehalt wurde der durch Enzym freigewordene anorganische Phosphor bestimmt.

Tabelle VII. (Versuchsanordnung.)

	A	B	C
1% Natr. Glycerophosphat (ccm)	20	0	20
Pufferlösung (ccm)	10	10	10
Fermentlösung (ccm)	10	10	0
Wasser (ccm)	0	20	10
Toluol (ccm)	1	1	1

Jeder Versuchskolben (A.B.C.) wurde dicht verschlossen und 24 Stunden lang im Brutschrank bei 37°C. aufbewahrt, was auch im vorigen Versuch ausgeführt wurde. Die Resultate sind in den folgenden Tabellen VIII u. IX zusammengestellt. Bei Versuch C

Tabelle VIII. (P. in mg.)

Nr. d. Versuche	Körpergewicht(g)	A	B	Differenz (A–B)	Abgespaltene P. (%)
1	230	8,20	0,33	7.87	34,6
2	190	8,31	0,30	8,01	35,2
3	210	9,56	0,36	9,20	40,5
4	230	7,48	0,33	7,15	31,4
5	190	11,05	0,33	10,72	47,1
6	190	7,75	0,30	7,45	32,8
8	260	8,23	0,30	7,93	34,9
8	280	8,45	0,30	8,15	35,8
9	270	8,53	0,30	8,20	36,0
10	315	8,88	0,33	8,55	37,6
Durchschnittswert (%)					36,6

Tabelle IX. (Kontroll.)

Nr. d. Versuche	Körpergewicht(g)	A	B	Differenz (A–B)	Abgespaltene P. (%)
1	230	11,97	0,33	11,64	51,2
2	230	12,47	0,36	12,11	53,3
3	210	12,38	0,30	12,08	53,1
4	210	13,05	0,33	12,72	59,3
5	230	9,83	0,33	9,50	41,8
6	175	8,49	0,33	8,16	35,9
7	250	9,90	0,36	9,54	41,9
8	300	8,65	0,33	8,32	36,6
9	280	8,78	0,33	8,45	37,2
10	250	9,25	0,33	8,92	39,2
Durchschnittswert (%)					44,9

wurde keine freie Phosphorsäure ermittelt. Somit sind keine Ergebnisse in den Tabellen angegeben

Aus den Tabellen VIII u. IX lässt sich ersehen, dass die Aktivität der Knochenphosphatase bei Zufuhr von Cholsäure im Vergleich mit der Kontrolle viel stärker herabgesetzt ist, und zwar beträgt der durch Knochenphosphatase aus Glycerophosphat abgespaltene Phosphor bei Zufuhr der Cholsäure durchschnittlich 36,6% und bei der Kontrolle 44,9%. Somit wurde die Menge der Knochenphosphatase von Meerschweinchen durch die Wirkung der Cholsäure um 18,5% herabgesetzt, wenn man die Menge der Knochenphosphatase der durch sie abgespaltenen Phosphorsäuremenge gleichsetzt.

4. Glycerophosphatase in der Leber und Niere bei experimentellem Stauungsikterus von Kaninchen.

Obwohl das Vorhandensein von Gallensäure im normalen Blut noch nicht sicher bekannt ist, so ist doch sicher, dass die Gallensäure durch enterohepatischen Kreislauf in die Leber gelangt und bei Stauungsikterus durch Rückfluss der Galle wenigstens im Blut zirkuliert. Bei Stauungsikterus soll nach vielen Autoren die Leber nekrotisch werden. Der Entstehungsmechanismus ist unter den Pathologen eine strittige Frage, aber Karasawa (1927) glaubt, dass diese stauungsikterischen Leberveränderungen der Giftigkeit der Gallensäure zuzuschreiben sind. In diesem Sinne ist es von grosser Bedeutung, die Wirkung der Glycerophosphatase in der Leber und Niere bei Stauungsikterus zu erforschen.

Methodik.

Als Versuchstiere wurden kräftige männliche Kaninchen benutzt, die mit Okara gefüttert worden waren. Zur Erzielung des experimentellen Stauungsikterus wurde der Bauch unter Laparatomie geöffnet, der Ductus choledochus jedesmal an zwei Stellen doppelt unterbunden, und wieder zugenäht. 48 Stunden nach der Operation wurde das Tier getötet, und ihm Leber und Niere entnommen. Die herausgeholte Leber oder Niere wurde im Porzellan mörser gründlich verrieben. Ein bestimmter Teil des Leber- oder

Nierenbreis wurde mit der zehnfachen Gewichtsmenge Chloroformwasser versetzt, dann 24 Stunden lang bei Zimmertemperatur stehen gelassen und nach Kay (1928) durch Watte langsam abfiltriert. Dieses getrübte Filtrat wurde in den Mengenverhältnissen der Tabelle X zum Versuch benutzt. Anderseits wurde dieser Organbrei unter Lüftung vollkommen bis zur Gewichtskonstante getrocknet, und das Verhältnis des trockenen Gewichtes zum feuchten Gewicht (Q) durch Wägung ermittelt. Dann wurden experimentell-stauungsikterischen Kaninchen 3 ccm einer 1%igen Natrium-Cholatlösung pro kg Körpergewicht subcutan verabreicht. 4 Stunden nach der Injektion wurde das Tier getötet, und ihm Leber und Niere entnommen. Diese Leber und Niere wurden unter genau gleicher Behandlung zum Versuch gebraucht. Als Kontrolltiere wurden Kaninchen, die 2 Tage gehungert hatten zum Versuch benutzt.

Tabelle X. (Versuchsanordnung.)

	A	B	C
1% Natr. Glycerophosphat (ccm)	20	0	20
Pufferlösung	10	10	10
10% Fermentlösung (ccm)	10	10	0
Wasser (ccm)	0	20	10
Toluol (ccm)	1	1	1

Als Substrat wurde β-Glycerophosphat verwendet. Die Pufferlösung bestand aus m/10 Glykokoll und m/10 Natronlauge, deren Ph 8,9 betrug. Bei der Kontrollversuchsreihe C wurde im Substrat keine Spur von anorganischem Phosphor nachgewiesen.

Der Glycerophosphatasegehalt der Niere und Leber von stauungsikterischen Kaninchen zeigt im Vergleich mit dem von normalen eine deutliche Abnahme, besonders bei der Niere, und diese Abnahme wird bei Stauungsikterus durch Zufuhr von Cholsäure aufs neue verstärkt, wie aus den Tabellen XI–XVI ersehen wird, in denen der Phosphatasegehalt mit der durch Hydrolyse des Glycerophosphates gespaltenen durchschnitlichen Phosphormenge angegeben wird. Bei experimentellem Stauungsikterum wurden

VERSUCH MIT DER NIERE.

Tabelle XI. (Kontrolle)

Nr. d. Versuche	Körpergewicht (g)	Nierengewicht (g)	A P. (mg)	B P. (mg)	Differenz (A–B)	Q	Abgespalt. P. (mg) pro g (trock. Gew.)
1	2050	12,0	13,79	1,58	12,21	4,47	51.58
2	2150	11,8	14,32	1,80	12,52	4,30	53,84
3	1600	10,0	12,94	1,66	11,28	4,26	48,05
4	2000	11,8	14,24	1,52	12,72	4,23	53,81
5	1740	10,0	13,30	1,57	11,73	4,48	52,55
6	1840	13,0	10,69	1,51	9,18	4,26	39,11
7	1800	11,2	13,63	1,58	12,05	4,30	51,82
8	1830	11,2	13,43	1,52	11,91	4,42	52,64
9	1870	11,7	13,16	1,58	11,58	4,18	48,40
Durchschnittswert							50,53

Tabelle XII. (Bei Stauungsikterus.)

Nr. d. Versuche	Körpergewicht (g)	Nierengewicht (g)	A P. (mg)	B P. (mg)	Differenz (A–B)	Q	Abgespalt. P. (mg) pro g (trock. Gew.)
1	2100	12,5	10,53	1,58	8,95	4,54	40,63
2	1650	12,0	10,58	1,66	8,92	4,62	41,21
3	2150	12,0	8,86	1,55	7,31	4,37	31,94
4	1750	11,5	11,16	1,55	9,61	4,56	43,82
5	2020	12,0	10,17	1,52	8,65	4,35	37,63
6	1860	11,4	11,44	1,47	9,97	4,40	43,87
7	1620	13,0	11,63	1,41	10,22	4,24	43,33
8	1960	10,8	10,33	1,50	8,83	4,25	37,53
9	1800	10,5	10,61	1,55	9,06	4,34	39,32
Durchschnittswert							39,92

Tabelle XIII. (Bei Stauungsikterus mit Cholsäure.)

Nr. d. Versuche	Körpergewicht (g)	Nierengewicht (g)	A P. (mg)	B P. (mg)	Differenz (A–B)	Q	Abgespalt. P. (mg) pro g (trock. Gew.)
1	1750	10,5	9,14	1,52	7,62	4,12	31,39
2	2280	13,2	9,69	1,50	8,19	4,49	36,77
3	1640	9,5	9,00	1,52	7,48	4,35	32,54
4	1860	11,2	9,39	1,47	7,92	4,24	33,58
5	1920	9,2	9,53	1,61	7,92	4,41	34,93
6	1700	10,4	11,14	1,66	9,48	4,30	40,76
7	1860	11,0	10,33	1,66	8,67	4,24	36,76
8	2180	11,5	10,57	1,57	9,00	4,37	39,33
9	1170	9,2	9,58	1,50	8,08	4,24	34,26
Durchschnittswert							35,59

VERSUCH MIT DER LEBER.

Tabelle XIV. (Kontrolle).

Nr. d. Versuche	Körpergewicht (g)	Lebergewicht (g)	A P. (mg)	B P. (mg)	Differenz (A–B)	Q	Abgespalt. P. (mg) pro g (trock. Gew.)
1	2050	40,0	4,82	1,30	3,52	3,28	11,55
2	2150	43,7	4,66	1,31	3,35	3,50	11,73
3	1600	33,5	4,70	1,26	3,44	3,34	11,49
4	2000	42,0	4,71	1,36	3,35	3,40	11,39
5	1710	41,9	4,71	1,30	3,41	3,66	12,48
6	1840	49,5	4,57	1,41	3,16	3,47	10,97
7	1800	49,2	5,00	1,26	3,74	3,73	13.95
8	1830	47,0	4,57	1,34	3,23	3,50	11,31
9	1890	41,0	4,34	1,34	3,00	3,40	10.20
Durchschnittswert							11,67

Tabelle XV. (Bei Stauungsikterus.)

Nr. d. Versuche	Körpergewicht (g)	Lebergewicht (g)	A P. (mg)	B P. (mg)	Differenz (A–B)	Q	Abgespalt. P. (mg) pro g (trock. Gew.)
1	2100	55,0	4,19	1,41	2,78	4,29	11,93
2	1650	47,0	3,91	1,47	2,44	4,54	11,08
3	2150	65,0	4,13	1,33	2,80	4,40	12,32
4	1750	42,0	3,30	1,44	1,86	4,52	8,41
5	2020	61,5	3,82	1,47	2,35	4,28	10,06
6	1860	55,0	3,02	1,22	1,80	4,48	8,06
7	1620	50,4	4,21	1,30	2,91	4,46	12,98
8	1960	51,8	3,88	1,30	2,58	4,35	11,22
9	1800	45,6	3,71	1,30	2,41	4,46	10,75
Durchschnittswert							10,76

Tabelle XVI. (Bei Stauungsikterus mit Cholsäure.)

Nr. d. Versuche	Körpergewicht (g)	Lebergewicht (g)	A P. (mg)	B P. (mg)	Differenz (A–B)	Q	Abgespalt. P. (mg) pro g (trock. Gew.)
1	1750	48,4	4,32	1,36	2,96	4,15	12,28
2	2280	61,5	3,10	1,30	1,80	4,57	8,23
3	1640	39,7	3,41	1,25	2,24	4,41	9,88
4	1860	61,0	3,60	1,44	2,16	4,28	9,24
5	1920	56,7	3,49	1,46	2,03	4,45	9,03
6	1700	53,8	3,78	1,57	2,21	4,59	10,14
7	1860	49,0	3,74	1,57	2,27	4,43	10,06
8	2180	52,8	4,10	1,47	2,63	4,46	11,73
9	1770	52,5	3,60	1,36	2,24	4,42	9,90
Durchschnittswert							10,05

durchschnittlich 39,32 mg Phosphor aus Glycerinphosphorsäure durch die Nierengewebe (pro g trocknen Gewichts) abgespalten, und bei experimentellem Stauungsikterus unter Zufuhr von Cholsäure durchschnittlich 35,59 mg Phosphor, während bei normalen Tieren durchschnittlich 50,53 mg Phosphor abgespalten wurden. Durch das Lebergewebe von stauungsikterischen Kaninchen (pro g trocknen Gewichts) wurden durchschnittlich 10,76 mg Phosphor aus Glycerinphosphorsäure unter genau gleichen Bedingungen wie bei der Niere abgespalten, während bei stauungsikterischen Kaninchen bei Zufuhr von Cholsäure durchschnittlich 10,05 mg Phosphor abgespalten wurden. Bei normalen Kaninchen wurden durch das Lebergewebe durchschnittlich 11,67 mg Phosphor abgespaltet.

Diese Resultate zeigen, dass der Glycerophosphatasegehalt der Leber und der Niere durch Rückfluss der Galle in den allgemeinen Kreislauf abnimmt. Diese Abnahme tritt bei der Niere viel stärker auf als bei der Leber. Aus den Ergebnissen scheint mir hervorzugehen, dass die Abnahme des Phosphatasegehaltes von Leber und Niere bei Stauungsikterus zum Teil der Wirkung der in den allgemeinen Kreislauf zurückgeflossenen Gallensäure zuzuschreiben ist.

Zusammenfassung.

1. Die Phosphorsäurespaltung aus Glycerinphosphorsäure durch Knochenphosphatase von Kaninchen wird durch Zusatz von Cholsäure hemmend beeinflusst; diese Hemmung tritt desto stärker ein, je mehr Cholsäure in der Versuchslösung enthalten ist.

2. Der hemmende Einfluss der Cholsäure auf die Glycerophosphatase tritt im Knochen viel stärker auf, als bei Leber und Niere.

3. Die phosphorsäurespaltende Wirkung der Knochenphosphatase aus Hexosemonophosphorsäure wird ebenfalls durch Zusatz von Cholsäure in der Versuchslösung gehemmt. Dieser hemmende Einfluss tritt desto stärker ein, je mehr Cholsäure in der Versuchslösung ist.

4. Die Wirkung der Knochenphosphatase wird bei Meerschweinchen durch subcutane Zufuhr von Cholsäure merklich her-

abgesetzt.

5. Der Glycerophosphatasegehalt der Niere und Leber von experimentell stauungsikterischen Kaninchen wurde im Vergleich mit dem von normalen Kaninchen herabgesetzt gefunden. Diese Herabsetzung des Phosphatasegehalts tritt vorzugsweise in der Niere ein.

6. Der durch experimentellen Stauungsikterus herabgesetzte Phosphatasegehalt von Leber und Niere wird durch Zufuhr von Cholsäure weiter aufs neue vermindert; auch diese Verminderung tritt in der Niere deutlicher auf als in der Leber.

7. Aus den oben erwähnten Ergebnissen kann man wohl den Schluss ziehen, dass als Ursache der durch Zufuhr von Gallensäure oder durch experimentellen Stauungsikterus vermehrten Ausscheidung der Phosphorsäure im Harn und in der Galle die Phosphorsäure des Phosphatides nicht in Betracht kommt, dass vielmehr der verminderte Phosphatasegehalt der Niere und der Leber bei Stauungsikterus wenigstens zum Teil auf die in den allgemeinen Kreislauf gelangte überschüssige Gallensäure zurückzuführen ist.

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ÜBER DIE GESCHLECHTLICHEN UNTERSCHIEDE DES OXYDATIONS- UND REDUKTIONS-VERMÖGENS IN DEN GEWEBEN.

(Zweite Mitteilung)

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Einleitung.

In einer früheren Mitteilung berichtete Verfasser über die geschlechtlichen Unterschiede des Oxydoreduktionsvermögens in den Geweben einiger Säugetiere unter Anwendung der Indophenoloxydasereaktion (chemische Vernonsche Methode) sowie der Entfärbungsreaktion auf Methylenblau. Er stellte dabei fest, dass das Oxydationsvermögen beim männlichen Tier stärker als beim weiblichen ist, während das Reduktionsvermögen sich umgekehrt verhält.

Zum Studium des Oxydationsvermögens werden heute hauptsächlich die Bestimmungsmethode der Gewebsatmung mittels Mikrorespirometer, die Barcroftsche Durchspülungsmethode sowie die Indophenoloxydasereaktion (chemische und histologische Methode) benutzt.

Die normalen Oxydasegranula sind schon von vielen Forschern (Gierke, Gräff, Klopfer, Lillie, Menten, Katsunuma, Fujiwara, Mori, u. a. eingehend behandelt worden. Neuerdings untersuchte Yasuda den Einfluss des Ovariums und des Hodens auf die Oxydasereaktion bei Kaninchen mittels der Gräffschen histologischen Methode. Leider haben alle diese Forscher die Menge der Oxydasegranula nicht nach quantitativen Gesichtspunkten bestimmt, wie überhaupt die Versuche über die geschlechtlichen Unterschiede dieser Granula recht spärlich sind.

Über den Sauerstoffverbrauch der Gewebe sind schon zahl-

reiche interessante Forschungen mittels der Warburgschen Methode ausgeführt worden. Aber die Autoren, welche Untersuchungen über den Sauerstoffverbrauch der Skelettmuskeln vornahmen, sind seltener, und man kann über dieses Problem nur einige Mitteilungen von P. Wels, Meyerhof und Hinwich, Iwazawa u. a. finden. Soviel Verfasser bekannt ist, gibt es fast keine Mitteilungen in Bezug auf die geschlechtlichen Unterschiede des Sauerstoffverbrauches der Skelettmuskulatur.

Neuerdings sind Untersuchungen von Champy, Caridroit und Szuman u. a. über den histologischen Einfluss der Kastration auf die Skelettmuskeln bei einigen Säugetieren veröffentlicht worden, aber die geschlechtlichen Unterschiede des Flächeninhalts der Muskelfaserquerschnitte haben diese Forscher nicht besonders eingehend diskutiert. Kiyohara und Ochi beobachteten diesbezügliche Unterschiede im *M. triceps femoris* von *Rana temporaria* und bestätigten, dass das weibliche Tier im Vergleich mit dem männlichen einen grösseren Flächeninhalt des Muskelfaserquerschnittes aufweist.

Davon ausgehend hat Verfasser beim Kaninchen die geschlechtlichen Unterschiede des Oxydationsvermögens mittels der Gierkeschen histologischen Methode, die des Flächeninhalts des Muskelfaserquerschnittes, und weiter die des Sauerstoffverbrauches mittels der Warburgschen Methode studiert.

Versuchsmethodik und Versuchsergebnisse

Als Versuchstiere wurden normale, gesunde und möglichst gleichgrosse (etwa 2 Kilogramm wiegende) Kaninchen gebraucht. Die Tiere wurden durch Verblutung aus der *A. carotis* getötet, woraufhin sofort der *M. gastrocnemius* (links) oder der *M. soleus* (links) herausgeschnitten wurde.

Zur Untersuchung der Indophenoloxydasereaktion wurden Schnitte (etwa 30 μ dick) des *M. gastrocnemius* unter Anwendung der Gefriermethode hergestellt, und weiter die Gierkesche Methode benutzt. Um die Menge der dabei durch das Indophenolreagens hervorgerufenen Granula quantitativ festzustellen, vergrösserte Verfasser in bestimmtem Grade (1:782) die Figur des

Muskelfaserquerschnittes mit Hilfe des kleinen Leitzschen Projektionszeichenapparats und kopierte dieselbe auf weissem Papier; daraufhin zählte er mit möglichster Genauigkeit die Granula in jedem Querschnitt. Dabei wählte er je 15 Muskelfaserquerschnitte für jedes Präparat aus und mass deren Flächeninhalt mittels des Amslerschen Planimeters je 10 mal für jede Muskelfaser, um möglichst einwandfreie Resultate zu gewinnen.

Zur Bestimmung des Sauerstoffverbrauches des Gewebes wurden bisher hauptsächlich dünne Schnitte benutzt; und diese Methode ist als die beste angenommen worden, weil diese Art Schnitt dem physiologischen Zustand am nächsten steht. Aber bei der Skelettmuskulatur ist diese Methode ungeeignet wegen ihrer technischen Schwierigkeit und der starken Schwankung der Resultate. Daher hat Verfasser die folgende Methode benutzt und dadurch konstantere Ergebnisse erzielt: Er setzt zu 2 g möglichst reinen Muskels 5 ccm Ringersche Lösung zu, zerreibt und vermischt den Muskel gründlich im Mörser und filtriert dann das

Tabelle I. (Kaninchen)

M-F-Querschnitt = Muskelfaserquerschnitt.
O-Granula = Oxydasegranula.

Geschlecht	Körpergewicht (g)	Flächeninhalt eines M-F-Querschnittes (qcm) (Vergrösserg. 1:782)	Zahl d. O-Granula in einem M-F-Querschnitt	Zahl d. O-Granula in 1 qcm Muskelfaser
♂	2000	56.9	728	12.8
♂	1950	55.9	777	13.9
♂	1950	74.1	925	12.5
♂	2050	65.8	803	12.2
♂	2000	70.7	889	12.6
♂	1900	52.3	610	11.6
♂	1950	61.8	698	11.3
♂	1950	62.1	764	12.3
♂	2000	60.4	861	12.6
♂	2000	62.3	689	11.1
	Mittelwerte	62.2		12.3

Tabelle II. (Kaninchen)

Geschlecht	Körpergewicht (g)	Flächeninhalt eines M-F-Querschnittes (qcm) (Vergrösserg. 1:782)	Zahl d. O-Granula einem M-F-Querschnitt	Zahl d. O-Granula in 1 qcm Muskelfaser
♀	2000	46.5	481	10.4
♀	2000	64.6	549	8.5
♀	1950	89.0	804	9.0
♀	1950	82.6	787	9.5
♀	2000	60.1	555	9.2
♀	1900	90.4	809	9.0
♀	2000	79.6	836	10.5
♀	1950	56.4	471	8.4
♀	1950	72.3	774	10.7
♀	2050	82.6	787	9.5
	Mittelwerte	72.4		9.5

Präparat durch reine Gaze. Aus dem 0.5 ccm Filtrat wird die Menge der Sauerstoffatmung pro Stunde nach dem Verfahren von Warburg ermittelt.

Wie in den Tabellen 1 und 2 zusammengefasst wird, ist die Menge der Oxydasegranula im Muskelfaserquerschnitt bei dem männlichen Kaninchen grösser als beim weiblichen.

Das weibliche Tier besitzt aber im Vergleich mit dem männlichen einen grösseren Flächeninhalt des Muskelfaserquerschnittes. Diese Resultate stimmen mit den Angaben von Kiyohara und Ochi fast überein.

Der Sauerstoffverbrauch sowohl des *M. gastrocnemius* als auch des *M. soleus* ist bei dem männlichen Kaninchen grösser als beim weiblichen. Vergleicht man die roten und weissen Muskeln, so ist der Sauerstoffverbrauch der roten immer grösser als der der weissen. Es ist interessant diese Ergebnisse mit denen von Battelli und Stern, Vernon, Date, Verfassers früheren Arbeiten und anderen zu vergleichen. Nach den Versuchen obiger Forscher ist bei Anwendung der Indophenoloxydasereaktion das Oxydationsvermögen der roten Muskeln stets stärker als das der weissen.

Tabelle III. (Kaninchen)

Geschlecht	Körpergewicht (g)	Sauerstoffverbrauch cmm O_2/0.5 ccm Std.	
		M. soleus	M. gastrocnemius
♂	2050	2.3	2.6
♂	1950	2.9	1.9
♂	2000	2 4	1.1
♂	2100	3.5	1.9
♂	2000	3.5	2.0
♂	Mittelwerte	2.9	1.9

Tabelle IV. (Kaninchen)

Geschlecht	Körpergewicht (g)	Sauerstoffverbrauch ccm O_2/0.5 ccm Std.	
		M. soleus	M. gastrocnemius
♀	2000	0.9	0.8
♀	2100	1.9	1.8
♀	2100	2.3	1.2
♀	2000	2.5	0.9
♀	1900	4.7	1.3
♀	1950	2.1	1.2
	Mittelwerte	2.4	1.2

Zusammenfassung.

Aus diesen Versuchen kommt Verfasser zu folgenden Schlüssen.

1. Beim männlichen Kaninchen ist die Menge bezw. die Zahl der Oxydasegranula im Muskelfaserquerschnitt grösser als beim weiblichen.

2. Der Sauerstoffverbrauch der Skelettmuskulatur erweist sich beim männlichen Kaninchen grösser als beim weiblichen.

3. Nach diesen Ergebnissen glaubt Verfasser folgern zu können, dass beim männlichen Kaninchen das Oxydationsvermögen stärker als beim weiblichen ist, was das Resultat seiner früheren Untersuchung weiter bestätigen würde.

4. Der Flächeninhalt eines Muskelfaserquerschnittes ist grösser beim weiblichen als beim männlichen Kaninchen.

Der Sauerstoffverbrauch der Muskulatur ist von Dr. K. Kiyohara in unserem Institut bestimmt worden, wofür ich ihm hiermit meinen herzlichen Dank aussprechen möchte.

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STUDIES IN HIPPURIC ACID.

I. A Method for the Determination of Hippuric Acid in Urine.

By

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I. Introduction.

Hippuric acid formation in an animal body, as an example of a simple synthetic process, has been considered as an interesting problem from the biological point of view, and there have been described a number of methods for the isolation and estimation of this substance. Since the hippuric acid formation has thrown a light upon certain phases of nitrogen metabolism, the study of hippuric acid has roused the special attention of investigators. However, owing to the difficulty of estimating it quantitatively, the study has made very slow progress. In this paper, a new simple and accurate method for the hippuric acid determination of urine is described.

II. Comparison of Methods.

We have attempted to criticise some existing methods and to make comparison of their reliability. The methods for the estimation are divided into the following three classes.

In the first class, hippuric acid is to be extracted from urine by some solvent and subsequently purified, preventing loss as much as possible, and weighed.

Bunge and Schmiedeberg method (1876), based on the principle above mentioned, has been widely employed in preference to all others.

Although this method is adequate for isolating hippuric acid

in pure form, it still needs to be much improved because of the complication in manipulation and inaccuracy of method. Folin and Flanders already stated that this method and its modifications have a defect that some part of hippuric acid may be decomposed by evaporating the urine which has been made alkaline.

There is another method in which hippuric acid is extracted from urine and hydrolysed with either alkali or acid, and the benzoic acid produced is isolated and weighed. Among these methods, the Dakin method (1910) is considered to be most accurate, but the manipulation is still complicated and tedious.

As for the methods in the second class, glycine obtained by hydrolysis of the hippuric acid extracted from urine is estimated by the formol titration method (Henrique & Sörensen, 1909; Quick, 1926) or by means of Kjeldahl method (Snapper & Laqueur, 1923; Griffith, 1926).

In the Henrique and Sörensen method, hippuric acid is repeatedly extracted from urine with ethylacetate while in the Quick method, hippuric acid is extracted with ether by continuous extraction apparatus. The hippuric acid thus obtained is hydrolysed with concentrated HCl, and the liberated glycine is to be determined by the well known formol titration method. Since, however, the amount of glycocoll liberated by splitting is too small to titrate (for instance, as in normal urine), the determination is not reliable.

In our experiments made on urine by the methods described above, we could not get a satisfactory result. Van Slyke (1913) reported a gasometric method for the determination of amino nitrogen of glycine, but it is also complicated. Blumenthal(1900) attempted to determine by means of the Kjeldahl method the nitrogen of the hippuric acid extracted from urine with ether, as he thought it difficult to get all the hippuric acid in crystalline form.

Snapper and Laqueur (1923) reported a method which might be considered a direct method for hippuric acid determination. The urine is extracted with a small portion of ethylacetate, and the extract, after the solvent has been removed by distillation,

is treated with alkaline hypobromite solution to destroy the urea carried over by the solvent. The residue is analysed by the Kjeldahl method for nitrogen from which hippuric acid can be calculated. However, the urea cannot be completely removed by this treatment, and empirical correction which has to be made for the undecomposed portion is a serious disadvantage inherent in this method.

Griffth (1926) recently found that ether is superior to ethyl-acetate for the extraction, since it extracts all the hippuric acid but practically no urea, and it is more comfortable when a continuous extraction apparatus is used.

Previous to the test of the Griffth method, we determined the nitrogen of various amounts of pure hippuric acid (Merk) used in our experiment by the Kjeldahl method (Table I).

Table I.

Hippuric acid used	Number of cc of 0.1 N acid neutralized by ammonia.	Hippuric acid recovered.	
gm.	cc.	gm.	%
0.01	5.5	0.098	980
0.01	1.8	0.031	310
0.15	9.2	0.164	109
0.15	9.0	0.160	106
0.16	9.5	0.169	112
0.125	6.9	0.122	97
0.50	28.1	0.502	100
0.50	27.8	0.499	99
0.50	28.2	0.504	100
0.50	27.3	0.488	97

As seen in Table I, it is impossible, unless there are pretty large amounts of hippuric acid, to get an accurate result by this method, and, in fact, as the contents of hippuric acid in urine are usually so small that the macro-Kjeldahl method is not applicable. We examined how much hippuric acid would be re-

covered from its solution of known concentration by extracting it with ether in a continuous extraction apparatus for an hour and by estimating its nitrogen by means of the Kjeldahl method, treating similarly as Griffth described. The results are shown in Table II.

Table II.

Hippuric acid used	Number of cc of 0.1 N acid neutralized by ammonia.	Hippuric acid recovered.	
mgm.	cc.	mgm.	%
80	3.75	67	83
500	23.5	420	84
160	7.25	129	80
160	8.2	146	91

Table II shows that by the procedure mentioned above the recovery of hippuric acid nitroen is only 83 to 91%.

Further, the hippuric acid in urine of normal rabbit is determined by Griffth method and the results are shown in Table III.

Table III.

No of rabbit	Amount of urine used for determination.	Number of ccm. of 0.1 N acid neutralized by NH_3	Hippuric acid obtained.
	cc.	cc.	mgm.
1	10.0	0.6	10.7
2	10.0	0.5	8.9
3	10.0	0.3	5.4
4	10.0	0.4	7.2

The following Table IV gives the results obtained by Griffth method with the same rabbit's urine to which a definite amount of hippuric acid has been added.

Table IV.

No. of rabbit	Urine used for determination	Hippuric acid added	N/10 NaOH	The found value	The theoretical value	Difference
	cc.	gm.	cc.	mg.	mg.	%
1	10.0	15	10.1	179	160.7	+11.3
2	10.0	15	9.7	172	158.9	+ 8.2
3	10.0	15	9.3	165	155.4	+ 6.1
4	10.0	15	1.4	167	157.2	+ 6.2

Such a great discrepancy between the found and the theoretical values, as indicated in Table IV, may be due to the contamination of some other nitrogenous substance carried over by ether.

In the third class of the methods, hippuric acid is decomposed by boiling the urine with either alkali or acid, and the benzoic acid is extracted and it is either weighed or titrated. Kingsbury and Swanson's modification (1921) of Folin & Flander's method (1912) was found to give a good result and to be convenient if the hippuric acid is contained in a pretty large amount in urine. But in the case where too small an amount of this acid is contained, as in dog's urine, this method becomes unreliable. Hryntschak (1912) has also reported the following method of hippuric acid determination.

After removal of urinary pigment by oxidation with potassium permanganate, the hippuric acid is extracted with ether, and hydrolysed. The benzoic acid, a decomposed product of hippuric acid, is purified and weighed. Although this method is found to be tolerably accurate, the manipulation is somewhat complicated.

Steenbock's method (1912) based on the following principle. Hippuric acid in urine is hydrolysed with NaOH, and urinary pigment is oxydised with H_2O_2. After the urine is acidified, it is extracted with ether. The benzoic acid in ether extract is sublimed in a suitable apparatus and weighed.

E. J. Wayne (1928), modified Stenbock's method to deter-

mine separately the hippuric acid from phenaceturic acid when both are simultaneously contained in urine.

We have tried to test the accuracy of Folin-Flander's method and Kingsbury-Swanson's methods and obtained satisfactory results when tested on pure hippuric acid solution. The results are given in Tables V-a and V-b.

Table V-a.
Folin and Flander's method.

Hippuric acid used	N/10 sodium ethylate	Hippuric acid recovered	
gm.	cc.	gm.	%
0.10	5.8	0.1038	103
0.10	5.6	0.1002	100
0.10	5.5	0.0985	98
0.10	5.6	0.1002	100
0.10	0.6	0.0107	107

Table V-b.
Kingsbury and Swanson's method.

Hippuric acid used	0.1 N sodium ethylate	Hippuric acid recovered	
gm.	cc.	gm.	%
0.080	5.0	0.0895	111
0.080	4.5	0.0806	100
0.016	1.0	0.0179	111
0.016	0.9	0.0161	100
0.160	9.0	0.1611	100

When, however, these methods were applied to urine, a good result could not be obtained, owing to the fact that the chloroform extract was deeply coloured with yellow by urinary pigment to

an undistinct end-point in the titration. The results obtained in our test experiment for these methods are indicated in Table VI-a (results of the experiment with human urine) and in Table VI-b (results of the experiment in which each 0.016 gm. of hippuric acid was added to the same human urine).

Table VI-a.
Human urine samples (by Kingsbury and Swanson's method).

No.	Urine	0.1 N sodium ethylate	Hippuric acid obtained
	cc.	cc.	gm.
1	50.0	2.0	0.03585
2	50.0	1.1	0.01969

Table VI-b.
Human urine+1 cc of 0.16% hippuric acid solution (by Kingsbury and Swanson's method).

No.	Urine	Hippuric acid added to the urine	0.1 N sodium ethylate	The found value	The theoretical value	Difference	
	cc.	gm.	cc.	gm.	gm.	gm.	%
1	50.0	0.0160	3.3	0.0591	0.0519	+0.0073	+12.2
2	50.0	0.0160	2.3	0.0412	0.0357	+0.0055	+13.4

Table VI-b shows that the found value differs from the theoretical one. Furthermore, in Folin-Flander's method, it takes 9 to 10 hours for the determination and in Kingsbury-Swanson's method also a pretty long time. In general, a method which needs hydrolysis or oxidation not only takes a long time for the estimation but also the manipulation is more complicated. Therefore as possible means of avoiding this F. J. Warth and N. C. Gupta (1928) proposed the following procedure:

Acidity the urine which has been hydrolysed with alkali. Make up to a definite volume with water and shake with a definite

volume of kerosene. An aliquot of the solvent is used for the determination of benzoic acid. By this means, the complicated extraction is not necessary any more, but it is obvious that in all the methods described above which belong to the third class, the error takes place by calculating total benzoic acid as hippuric acid, when free benzoic acid or other forms of conjugated benzoic acid are present in urine.

However, the majority of the methods above indicated stand on the basis on hydrolysis of hippuric acid. In case an acid is employed for hydrolysis, amino acid is determined while in the alkaline case, benzoic acid is estimated. So far as we experimented, hydrolysis of hippuric acid did not bring forth a satisfactory result. At any rate, these methods are not convenient as they require complicated and inaccurate hydrolysis or oxidation in their manipulation.

The sole method in which it was attempted to isolate hippuric acid from urine without hydrolysing and to titrate directly for its determination was W. A. Cate's modification (1901) of Bunge and Schmiedeberg's method. Yet this method was not convenient for the isolation of hippuric acid.

Since the direct titration of the acid seems to offer the best quantitative method for hippuric acid, the most favorable condition for isolation of this acid from urine was studied.

It was already found by Griffth and Quick that ether was superior to ethylacetate for the extraction of hippuric acid, since it could extract all the hippuric acid in urine, but practically no other urinary constituents. We have also ascertained the fact that hippuric acid in urine was quantitatively extracted with ether. In the following paragraph, our method for the determination of hippuric acid in urine will be described.

III. Principle.

After acidifying the urine with H_2SO_4, the urine is extracted with ether by means of Kumagawa-Suto's extraction apparatus for fluid. The ether extract is rinsed with petroleum ether to get rid of the contamination of benzoic acid, and then dissolved

in hot water and titrated with N/10 sodium hydroxide using phenolphthalein as an indicator. The amount of hippuric acid will be calculated from the number of ccm of N/10 NaOH.

IV. Procedure.

20 cc of protein free urine is used (when the amount of hippuric acid is large, a proportionately small quantity of urine is to be employed).

After acidifying urine with 1 cc. of 5n H_2SO_4, the urine is extracted with ether by Kumagawa-Suto's extraction apparatus for fluid at 60–70°C for 3 hours. By evaporating the ether, the hippuric acid usually remains in crystals at the bottom of the flask. Wash these crystals with a small portion of petroleum ether, immersing the flask in a water bath at about 85°C for 3 minutes.

Then the petroleum ether is filtered off through an asbestos filter every time and the filter is washed out into a small beaker with a small portion of hot water several times. The hippuric acid remaining in the flask is dissolved with approximately 10 cc of hot water, to which the wash water is added and the mixture is titrated with N/10 sodium hydroxide using phenolphthalein as an indicator. 1 cc. of N/10 NaOH is equivalent to 0.0179 gm. of hippuric acid. It is proved that only a negligible amount of sulphuric acid used for acidifying the urine goes into the ether during the extraction.

The urinary pigment usually does not exert any influence upon the end-point of the titration. If, however, the urine is deeply coloured, it is better to dilute with water or to use a small quantity of urine. Urine should not be treated with charcoal for decolorising because it will bring forth some error in titration value. It was found also that a trace of protein in urine gives rise to the formation of emulsion in the supernatant ether layer. Yet it is successfully eliminated by adding 1.0 to 5.0 cc. of 1% sod. tungstate to the urine. Neither the precipitated protein tungstate nor an excess of tungstic acid interferes with the extraction of hippuric acid. If the urine contains a large amount of

lactic acid it will be better to use $KMnO_4$ for its oxydation previous to the extraction. The details will be seen in the next paper.

V. Experiment.

When the method as outlined was tested on pure solution of known concentration of hippuric acid, it was found that hippuric acid will be recovered in 100% within one and half hours. The results are shown in Table VII.

Table VII.

No. of experiment	Hippuric acid used	Hours for extraction of hippuric acid	Number of cc. of 0.1 N NaOH needed	Hippuric acid recovered	
	gm.	hour	cc.	gm.	%
1	0.0320	1	1.5	0.0268	83
2	0.0320	1½	1.8	0.0320	100
3	0.0160	1½	0.9	0.0160	100
4	0.0320	2	1.8	0.0320	100
5	0.0320	3	1.8	0.0320	100

The following data were obtained from each specimen of human urine (see Table VIII).

Table VIII.

No. of urine	Urine used	Hours for extraction of hippuric acid	Number of cc. of 0.1 N NaOH	Hippuric acid obtained
	cc.	Hour	cc.	gm.
1	20.0	2	0.8	0.0143
2	20.0	2	0.9	0.0161
3	20.0	2	0.7	0.0125
4	20.0	2	0.75	0.0134
5	20.0	3	0.9	0.0161

As seen in Table VIII, the amount of hippuric acid in 20 cc. of human urine is from 0.0125 to 0.0161 gm. To test the rate of recovery of the hippuric acid from the urine by this method, we added 16 mg. of hippuric acid to the same specimens of the urine above mentioned and estimated. The results are given in Table IX.

Table IX.

No. of urine	Urine used	Hippuric acid added	Hours for extraction of hippuric acid	Number of cc. of N/10 sodium hydroxide	Hippuric acid recovered	
	cc.	gm.	Hour	cc.	gm.	%
1	20.0	0.016	2	1.6	0.0286	95
2	20.0	0.016	2	1.7	0.0304	95
3	20.0	0.016	2	1.7	0.0304	95
4	20.0	0.016	3	1.8	0.0322	100
5	20.0	0.016	4	1.8	0.0322	100

As shown in Table IX, 95% or 100% of added hippuric acid is recovered after extraction for 2 or 3 hours respectively.

VI. Summary.

1. An accurate, simple, and direct method for the determination of hippuric acid in urine has been described.

2. It can be determined easily with satisfactory results.

3. The determination can be completed approximately within 3 hours.

4. The hippuric acid added to the urine can be quantitatively recovered by ether extraction within 2 to 3 hours.

5. Kumagawa-Suto's extraction apparatus for fluid is convenient for the extraction.

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STUDIES IN HIPPURIC ACID.

II. A Method for the Determination of Hippuric Acid in Blood.

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I. Introduction.

Previously the author has published a paper on a new method for the determination of hippuric acid in urine. This method will be recommendable for general use because of its simple manipulation and its satisfactory results. The determination can be completed within approximately 3 hours. The author also attempted to apply this method for the determination of hippuric acid in blood and also obtained a good result.

So far as we know, there are described only 2 methods for the estimation of hippuric acid in blood. One of them is Friedman and Taschau's method (1911) and the other is Kingsbury's (1915). The principle of the former is that hippuric acid in blood is extracted by some solvent and then purified and weighed ,but, as many authors have already stated, it is used very seldom on account of its having but little ability to recover 75 per cent of the hippuric acid added.

As the latter method is to determine hippuric acid as total benzoic acid after hydrolysis, it should not be employed when free benzoic acid and any other combined benzoic acid occur in blood. Furthermore, the end -point in titration is not distinctive. In the present paper a new method for the determination of hippuric acid in blood is described.

II. Principle.

The non-protein blood filtrate is prepared by Haden's modifi-

cation and the lactic acid in it is quantitatively split by potassium permanganate to aldehyde (Ernst, 1930, Lehnartz (1928); Zacharias and Laszlo (1927)) and evaporated off. In this case, hippurate remains as hippuric acid, which is extracted with ether in continuous extraction apparatus. The ether soluble substances intermixed in the ether extract other than hippuric acid are removed by petroleum-ether. Pure hippuric acid thus obtained is dissolved in hot water and titrated with alkali.

III. Procedure.

In the present experiment, for the purpose of removing protein from blood, we employed Haden's modification (1923) of Folin and Wu's method, which has the following advantages as compared with the original, i.e., (1) simple in manipulation, (2) easy in filtration, (3) producing large amounts of the filtrate.

To prevent coagulation of blood, 20 mg. of potassium oxalate is used. A measured quantity (5 to 15 cc.) of oxalated blood is transferred into a flask having a capacity of fifteen to twenty times the volume of the blood taken.

The blood with eight volumes of N/12 H_2SO_4 is laked by adding very slowly from a graduated biurette and shaking constantly. Whereupon one volume of 10 per cent sod. tungstate solution is added and mixed. The mouth of the flask is closed with a rubber stopper and it is shaken well. When it is allowed to stand 5 minutes, the color changes from bright red to dark brown.

The whole of the mixture is poured on a filter large enough to hold it all and covered with a watch glass. If the filtration is done as described, the very first portion of the filtrate will be clear as water, and no re-filtering is necessary and the filtrate does not contain even a trace of protein.

In the case where the protein of the blood is removed completely, pH of the filtrate will be 2.8 or more acid. The blood filtrate equivalent to half the volume of the blood taken is used for the determination. Previous to the estimation, the lactic acid which will sometimes appear in the blood filtrate has to be split off

by means of the following manipulation: Transfer the blood filtrate for the determination into a small beaker or Erlenmeyer flask and boil gently on an asbestos plate. From a biurette run into it drop by drop, N/10 $KMnO_4$ until a faintly but permanent brown color takes place. Here the lactic acid is completely oxydised into aldehyde and evaporated off. Table I shows that the lactic acid is quantitatively decomposed by $KMnO_4$ while the hippuric acid is left quite intact.

Table I.

No. of experiment	Lactic acid used	Hippuric acid added as Hippurate	ccm. of N/10 $KMnO_4$ required for decomposition of lactic acid	ccm. of N/50 NaOH required for the neutralisation of the acid of the ether extract	Recovery of hippuric acid
	gm.	gm.			gm.
I	0.002	—	0.9	0.06	—
II	0.002	—	1.0	0.04	—
III	0.002	—	0.8	0.06	—
IV	0.002	0.0083	1.0	2.4	0.0085
V	0.002	0.0083	0.9	2.35	0.0084
VI	0.002	0.0083	0.9	2.35	0.0084

Then the solution is extracted continuously with 100–150 cc. of ether by Kumagawa-Suto's apparatus for 3 hours. After evaporation of ether, the dry crystalline residue remaining in the flask is heated two to three times with a small quantity of petroleum ether at about 85°C for 3 minutes in water bath to remove the last traces of such petroleum ether soluble substances as fat, fatty acid, and oxybutylic acid which may be intermixed in it. Petroleum ether is filtered off through an asbestos funnel each time and the filter is washed with a small portion of hot water. The hippuric acid remaining in the flask is dissolved with 10 cc. of hot water, and this solution is mixed with the wash water of the filter mentioned above. After cooling, it is titrated with

N/50 sodium hydroxide, using one drop of alisalin as an indicator. One cc. of N/50 NaOH is exactly equivalent to 0.00357 gm. of hippuric acid.

Calculation. If N represents the number of cubic centimeters of N/50 NaOH and b the number of cubic centimeters of blood used, calculation would be as follows: $\frac{N \times 0.00357 \times 200}{b}$ = gm. of hippuric acid per 100 cc. of blood.

IV. Accuracy of the Method.

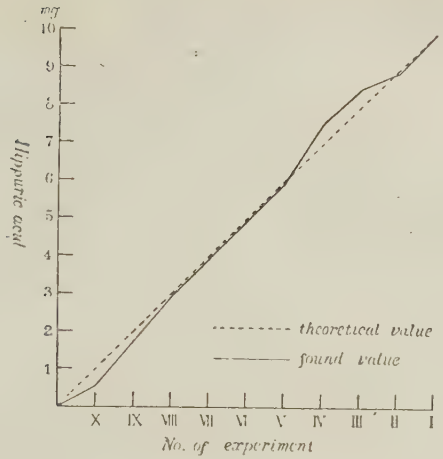
The accuracy of the method as outlined has been tested on the ox blood to which was added the solution of known concentration of hippuric acid. When over 3 mg. of hippuric acid were added to 10 cc. of the blood, it gave a satisfactory result, showing recovery, over 98% of the theoretical value. The data are shown in Table II and graphically in Fig. 1.

Table II.
Recovery of Hippuric Acid added to Blood.

No. of experiment	Blood	Hippuric acid added	Hippuric acid	
			Found	Recovered
	cc.	gm.	gm.	%
I	10.0	0.010	0.01000	100.0
II	10.0	0.009	0.00893	99.3
III	10.0	0.008	0.00849	103.0
IV	10.0	0.007	0.00756	104.0
V	10.0	0.006	0.00599	99.8
VI	10.0	0.005	0.00499	99.8
VII	10.0	0.004	0.00393	98.3
VIII	10.0	0.003	0.00293	97.7
IX	10.0	0.002	0.00175	87.5
X	10.0	0.001	0.00057	57.0

The purity of hippuric acid (Merk) used for the experiment was examined by determining its nitrogen content and its melting

Fig. 1



point. As will be seen in Table III, it was proved that the hippuric acid is chemically pure.

Table III.

No. of experiment	Hippuric acid	Nitrogen of hippuric acid		
		Theoretical value	Found value	Recovered
	gm.	gm.	gm.	%
1	0.5	0.0394	0.03934	99.7
2	0.5	0.0394	0.03948	102.0
3	0.5	0.0394	0.03892	98.7
Average	0.5	0.0394	0.03925	99.6

The melting point of the hippuric acid used for the experiment showed 187°C.

In the experiment with ox blood to which no hippuric acid was added, 0.01 cc. to 0.04 cc. of N/50 NaOH was always enough to neutralize the acid contaminated in the ether extract of the blood filtrate. Considered from the fact that the acidity has nothing to do with the amount of blood used, it may be due

to a minute amount of sulphuric acid dissolved in the ether used for the extraction. In fact, we were able to verify that it was true. When 50 cc. of distilled water acidified with H_2SO_4 in the same manner as described above, are extracted with ether, the ether extract shows an acidity equivalent to about 0.01–0.05 cc. of N/50 NaOH. At any rate, in case a tolerably large amount of hippuric acid is present in the blood, it is entirely negligible. It will therefore be considered that the ox blood has practically been free from hippuric acid. The results are shown in Table IV.

Table IV.

No. of experiment	Blood	Filtrate	N/50 NaOH
	cc.	cc.	cc.
1	1.0	4.0	0.01
2	2.0	10.0	0.02
3	3.0	15.0	0.02
4	4.0	20.0	0.01
5	5.0	30.0	0.02
6	10.0	50.0	0.01
7	20.0	100.0	0.04

V. Conclusions.

1. A simple and an accurate method for the determination of hippuric acid which is added to the blood has been described.

2. The manipulation is just the same as that in the case of urine with the exception of preparing a non-protein blood filtrate and of decomposing the lactic acid by potassium permanganate.

3. Ox blood is practically free from hippuric acid.

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STUDIES IN HIPPURIC ACID.

III. On the Permeability of Hippuric Acid to Red Blood Corpuscles.

By

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(Received for publication, March 18, 1932)

It is a general assumption that hippuric acid is synthesized in the kidney and immediately excreted in urine without circulating through the blood stream, and that in normal blood, there can be found only a trace of this acid which can hardly be determined. Recently Snapper and Grünbaum (1924) stated as follows: "Vier Stunden nach der Eingabe von 3×5 g Benzoes. Natr. fanden wir also:

1. Bei 3 Menschen mit normaler Nierenfunktion keine oder nur Spuren Hippursäure im Blut.

2. Bei 3 Patienten mit beginnender Niereninsuffizienz, d.h., deutlicher Urearetention und leicht verzögerter Hippursäure ausscheidung, kleine Mengen Hippursäure im Blut."

As a number of investigators have already stated, it has been shown in our experiment also that no hippuric acid is detected in the blood of normal animals such as man, ox, horse, dog, cat, rabbit, and guinea pig. We could not find any hippuric acid either in the blood of rabbits to which a large amount (1.0 gm per kilo) of sodium benzoate was given orally.

We investigated the duration of the presence of hippuric acid in the blood stream when it is injected intravenously in the rabbit. For this investigation, 150 ccm of M/100 sodium hippurate (2.1 gm. of hippurate was dissolved in 150 ccm of physiological salt solution) was injected intravenously. Then the amount of hippuric acid in the blood which had been remaining at various times after the injection were estimated. As will be seen in Table I,

it is clear that the hippuric acid injected intravenously in the rabbit is found in the blood even 24 hours after the injection, although there is only a trace of it.

Table I.

Rabbit No.	Body Wt.	Amount of Blood employed.	Amount of sodium-hippurate	Hippuric acid in Blood		
				Before Injecton	After Injection	
	gm.	ccm.	gm.	gm.		gm.
I	2650	8.0	2.1	0	at once.	0.0136
II	2756	8.0	2.1	0	20 mins.	0.0138
III	2645	8.0	2.1	0	2 hrs.	0.0057
IV	2768	8.0	2.1	0	3 hrs.	0.0036
V	2675	8.0	2.1	0	6 hrs.	0.0075
VI	2780	8.0	2.1	0	9 hrs.	0.0061
VII	2675	8.0	2.1	0	12 hrs.	0.0028
VIII	2770	8.0	2.1	0	24 hrs.	0.0007

As is obvious from the fact just mentioned above, it can be investigated whether hippuric acid in an animal body is permeable to red blood corpuscles or not. We injected the sodium hippurate dissolved in physiological saline solution to the rabbit intravenously, and the blood was taken from the ear vein at one to nine hours after the injection. Potassium oxalate powder was employed to prevent clotting of the blood. The blood was centrifuged and the amounts of hippuric acid in the plasma and red corpuscles were determined separately. The corpuscles had been washed previously with physiological salt solution two or three times to remove some hippurate which might have been contaminated physically with the corpuscles. The results are shown in Table II.

As is seen in Table II, it was recognised that the sodium hippurate injected into the blood stream was found to be impermeable to red blood corpuscles at least within 9 hours after the injection. Further experiment upon the permeability of hippuric acid to blood corpuscles in vitro was carried out. For the experiment, 10 cc. each of blood of rabbit and of ox was used,

TABLE II.
Distribution of Injected Hippuric Acid in Blood (Rabbit).

No. of animal	Body Wt.	Amount of sodium hippurate injected	Intervals between injection and bleeding	Blood amount employed	Hippuric Acid in	
					Plasma	Corpuscles
	gm.	gm.	hrs.	cc.	gm.	gm.
1	2350	0.198	1	10.0	0.00714	0
2	2750	0.220	3	10.0	0.01214	0
3	2785	0.220	6	10.0	0.00393	0
4	2365	0.198	9	10.0	0.00286	—
5	2850	0.220	9	10.0	—	0

and 5 mg. each of hippuric acid in solution was added to the respective blood sample. After allowing to stand for several hours at room temperature or in an incubator, the hippuric acid in the whole blood, in the corpuscles and in plasma was determined separately by the author's method (1932). The data are summarised in Tables III and IV.

TABLE III.
Distribution of Added Hippuric Acid in Blood

No. of animal	Animals	Blood amount used	Amount of hippuric acid used	Intervals between injection and bleeding	Hippuric acid in		Hippuric acid recovered
					Corpuscle	Plasma	
		cc.	gm.		gm.	gm.	%
1	Rabbit	10	0,005 (hippuric acid)	Immediately	0	0.00499	100.0
2	„	„	„	6 hrs. (at room temp.)	0	0.00492	98.0
3	Ox	„	„	Immediately	0	0.00478	96.0
4	„	„	„	6 hrs. (at room temp.)	0	0.00489	98.0
5	„	„	„	24 hrs. („)	0	0.00490	98.0
6	Rabbit	„	0.0083	12 hrs. („)	0	0.00840	101.0
7	„	„	„	24 hrs. („)	0	0.00820	98.7

Table IV.

No. of experiment	Animal	Blood amount used	Amount of hippuric acid added	Hours incubated	Hippuric acid in		Hippuric acid recovered
					Plasma	Corpuscles	
		cc.	gm.		gm.	gm.	%
1	Rabbit	10	0.0083	6	0.0078	0	93.9
2	„	„	„	12	0.0082	0	98.7
3	„	„	„	24	0.0080	0	96.3

In Tables III and IV it is evident that the hippuric acid added to the blood sample of ox and of rabbit, is not permeable into the blood corpuscles within 6 to 24 hours at room temperature or likewise in an incubator.

Conclusions.

1. The hippuric acid cannot be found in normal blood of such animals as man, ox, horse, cat, dog, guinea pig and rabbit. It cannot be detected even in the blood of a rabbit to which sodium benzoate is given orally.

2. The hippurate intravenously injected to rabbit is found in the blood even 24 hours after the injection.

3. The hippurate injected intravenously seems to be impermeable to the red blood corpuscles at least within 9 hours after the injection.

4. The hippuric acid added to the blood sample in vitro, is not permeable to the red blood corpuscles within 24 hours at room temperature.

5. The same results were obtained by the experiment in an inocubator.

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NUTRITIONAL STUDY ON DERMESTID BEETLES.

I. The Chemical Composition, and Especially the Nature of the Ether Extract of Beetles.

By

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The Dermestid beetles are well known to be noxious for the preservation of biological materials of high protein and low moisture content, devouring chiefly dried meat or fish, soy-bean preparations, leather and fur, etc. In our country several species of the insects have been described (Yokoyama, 1930-31), amongst which most common ones in Kyoto are *Dermestes coarctatus*, Harold, and *D. vulpinus*, Fab. In order to discuss the means of control of these insects, we must naturally know their biology and physiology beforehand.

According to the investigation by one of the writers (Sinoda, 1931) on the nutrition physiology of the silkworm, the chief source of nutrition for the silkworm is not carbohydrate, but the protein in the mulberry leaves, and so the protein metabolism is an important factor in order to know something about the nutrition physiology of insects even if they are of herbivorous habits. The Dermestid beetles offer the most suitable subject for the study of protein metabolism, both because they devour only proteinous diet, and also because they are very easy to rear in the laboratory.

Here the writers wish to express their hearty thanks to Prof. S. Komatsu and Prof. T. Kawamura for their advice and encouragement during the experiment. Dr. Y. Iwamoto of Osaka Municipal Institute of Technical Research gave them valuable criticisms concerning the nature of fatty acids, and Dr. T. Yokoyama of Imperial Sericultural Experiment Station, Tokyo, has

kindly identified the insects used. To these gentlemen the writers owe sincere gratitude. Acknowledgements are due also to Tôshôgû Tercentenary Fund for defraying the cost of experiment.

Both *Dermestes coarctatus* and *D. vulpinus*, which were used in the experiment, are very common in Kyoto. They were collected from warehouses keeping dried fish preparations (Kyoto, Miyazu) or dried silkworm cocoons (Ayabe). In autumn and winter the latter species is much more abundant than the former. However, the relative numbers of these two kinds collected varies with the locality and with the season as will be seen in the following table. These facts may be explained by the difference in the resistance against cold.

Table I.

The Number Ratios of the Materials.

Miyazu, 27/VIII/1930.		Kyoto, 28/VIII/1931.	
D. coarctatus	65	D. coarctatus	580
D. vulpinus	10	D. vulpinus	4
Mixed larvae	192	Mixed larvae	50
Ayabe, 3/IX/1931.		Laboratory, 17/XI/1931.	
D. coarctatus	70	D. coarctatus	41
D. vulpinus	4	D. vulpinus	35
Mixed larvae	2,210	Mixed larvae	113
		Pupae	19

These beetles were brought to the laboratory and after being starved for two days were fed with a standard meal, Katsubushi, the dried preparation of Bonito fish, *Euthynnus vagans*, manufactured in 1929 at Shuku-ura, Miye-ken. The chemical composition of dried bonito fish used for the meal was determined as follows:

Table II.

The Composition of Katsubushi, or dried Bonito Fish.

Moisture	14%
Total Nitrogen	14.3
(for dry basis)	16.6
Ether extract	5.5
(do.)	6.4

Physical Constants of the ether extract and its free fatty acid are:

	A.V.	I.V.	S.V.	N.V.	R.M.V.
Oil	129.3	137.3	227.1	—	0.9

	Pol. V.	Unsap. Mat.	clar. pt.	density $\frac{30°}{4°}$	$n_D^{30°}$
Oil	—	9.6	33°	1.010	1.4790

	A.V.	I.V.	S.V.	N.V.	R.M.V.	Pol. V.
F.f.a.	—	154.0	—	177.8	—	—

	Unsap. Mat.	m. p.	clar. pt.	density $\frac{80°}{4°}$	$n_4^{30°}$
F.f.a.	—	42°	—	—	—

F.f.a.: Free fatty acids.

In the analysis of larvae, as it is quite difficult to identify larval forms, the mixed larvae of both species were analysed together without separation.

Table III.
Moisture.

Stage	Date	Mean Weight mg.	Moisture	N.B.
Larva	6/IX	17.0	64%	Mixed
	18/IX	39.9	60	„
	6/IX	50.6	56	„
	13/IX	59.0	56	„
	23/IX	66.4	54	„
Pupa	18/IX	55.9	52	„
Adult	6/IX	34.2	54	*D. coarctatus*
	23/IX	46.2	54	„
	13/IX	49.0	55	„
	6/IX	32.9	55	*D. vulpinus*

	per day per 1 indiv.	Moisture
Faeces of Larvae	4.6 – 6.9 mg.	12–15%
„ of Adults	1.8	10–18

The moisture content in the insects decreases, though but little, as the insects grow old, but generally remains about 55%, viz. four times that of the diet. It is, therefore, highly probable that the metabolic water is retained in the animal body to maintain such high percentage of moisture. At the same time it must be pointed out that the moisture content of 55% is an extraordinarily low value compared with that of other insects; Coleoptera and Orthoptera contain between 60 and 70% of water and Lepidoptera more than 80%. Concerning whether these differences are due to their phyllogenetic positions, or to their food habits, further study is being made.

Total nitrogen decreases with age, keeping some parallerism with the moisture content. The amount of lipoid and chitin nitrogen calculated by the usual methods was found to be 0.2%, 0.6% in 7.5% total nitrogen for the larvae and 1.5% and 2.5% in 8.2% total nitrogen for the adults respectively. Lipoid nitrogen recalculated against the etherial extract is 0.53% for larvae and 3.2% for adult. The fact that the total nitrogen content in the insect is very poor when compared with the total nitrogen of the food (14%) and that of faeces (19%), is noteworthy, especially when we consider these facts with the content of ether extract of the insect.

The ether extract of the larvae reaches as high as 47% while that of the food is only 5.5%, and that of faeces is between 2 and 5%. The interesting thing is that the oil is almost exclusively localized in the adipose tissue, and is not distributed (in measurable quantities) in other tissues. When one dissects the insect with freezing microtome (without fixing it) and stains with Sudan III, the large fat bodies, which are stained orange red, may be seen at both sides of the alimentary tract.

Such an enormous accumulation of oil in the insect body can be explained in two ways: either the insect has absorbed the oil of the food selectively, or the oily matter has been converted from proteins (the chief constituent of the food) by desamination.

TABLE IV.

Chemical Compositions of the Beetle.

Sample	Date	Mean weight	For Dry Basis		
			Total Nitrogen	Ether Extract	Chitin
Larvae	27/XI	8.0 mg.	10.3	—	—
„	27/XI	16.9	9.4	—	—
„	29/VIII	46.3	—	39.0	—
„	21/XI	48.9	7.4	—	—
„	6/IX	50.6	—	40.5	7.7
„	23/XI	56.3	7.5	—	—
„	23/XI	64.1	6.9	—	—
„	30/VIII	66.5	—	46.7	—
Adults (D. coarctatus)	6/IX	34.2	—	21.1	2.9
„	21/XI	36.6	8.2	—	—
(D. vulpinus)	23/XI	45.9	8.4	—	—
„	23/XI	40.0	8.2	—	—

Chemical Compositions of the Faeces of the Larvae.

	Date	Moisture	For Dry Basis	
			Total Nitrogen	Ether Extract
Larvae	13/IX	12	—	—
	18/IX	15	—	—
	23/XI	10	19.4	—
	23/XI	10	19.3	—
	4/IX	15	—	2.2
Adults	23/IX	18	—	—
	13/IX	10	—	—

Indeed, both processes are seen in many animals (cf. Grünn, 1929), and to settle the question in this case, an intimate study of the nature of the insect oil must be studied. Especially because in Dermestid beetles, the accumulation of carbohydrates (esp. glycogen) seems to be but little compared with that of oily matter (if one calculates the nitrogen content of dry insect body free from chitin and oil, it is more than 15%), the solution of the oil formation problem is at the same time the solution of the problem of the protein metabolism in these beetles.

In Table V are shown some physical and chemical constants of oils from the beetle, and of a Lamellicornian one, which is absolutely of physophagous habit. These characteristics differ considerably from those of the oil of Katsubushi, the food, whereas the difference is but little between carnivorous and herbivorous beetles, viz. *Dermestes* and *Cetonia*. The resemblance of the characteristics of oils from insects belonging to the same, or similar, orders may be seen in other species also. (cf. Grünn, vol. 2, p. 490 ff.). The results seem to support the proteinogenous origin of the oil, but further study is going on to settle the question.

In order to see more precisely the chemical relation between the oils of insects and the food materials, some physical and chemical constants of free fatty acids of two oils were examined with the results shown in Table V.

There are 33% solid fatty acids in the total acids of the oil from the larvae (separation after Twitchell), which were assumed from the melting point and the neutralisation value to consist of 38% stearic and 62% palmitic acids (de Visser, 1898).

Of liquid fatty acids giving dioxy-stearic and sativic acids by oxidation with $KMnO_4$ in an alkaline media (after Hazura) the presence of oleic and linolic acids was confirmed and the presence of linolenic acid is certain from the bromination experiment.

Since the latter acid is not yet isolated from oils of marine fishes so far as the writers know, it was assumed to be formed in the beetle body during the course of metabolism.

The presence of a trace of higher unsaturated acids (such as clupanodonic acid) is indicated by the high melting point of the

TABLE V.

Physical and Chemical Characteristics of the oils *of the Beetles.*

Physical Constants of the Oil.

Sample	Date	Colour	Consistency	Clarif. pt.	d_4^{30}	n_D^{30}
A (Larvae)	23/IX/'30	Dark brown	oil	—	0.9146	1.4652
B „	8/IX/'31	Darty gr. yell	Semisolid	ca. 54°	0.8911 (55°)	1.4657 (1.4556, 55°)
C „	24/VIII/'31	Yellow	oil	ca. 45°	0.9069	1.4643
D (Adult)	30/VIII/'31	Dark brown	Heavy oil	—	0.9109	1.4658
E „	24/VIII/'31	Pale yellow	oil	—	—	1.4644
F Faeces (Larvae)	4/IX'31	Dark brown	Paste	ca. 35°	—	1.4854
G Cetonia brevitarsis Lewis (Adult)	29/VIII/'31	Greenish Brown	oil	—	0.9045	1,4645

Chemical Constants of the Oil.

Sample	I.V. (Wijs)	Acid V.	R.M.V.	Sap. V.	Pol. V.	Unsap. Matt. (Spitz & Hönig)
A	90.7	2.5	—	198.0	—	2.9
B	89.4	19.5	0.2	190.4	0.5	2.1
C	79.3	34.7	—	199.0	—	1.7
D	85.7	21.0	—	188.9	—	5.3
E	79.5	—	—	—	—	—
F	144.4	—	—	—	—	—
G	58.0	20.6	—	194.9	—	4.2

Chemical Constants of the Free Fatty Acids of the Oil of Larvae (B).

Sample	m. p.	N. V.	I. V.
Total fatty acids	41°	212.1	93.7
Solid acids	55°.9	211.7	11.8
Liquid acids	—	203.1	110.9

polybromide, but further research is required to settle this problem. Moreover the fact that the beetle oil shows very low iodine value compared with that of the food, Katsubushi, seems to support the hypothesis that the oil in the insects is partly synthesized from protein substances of the food. Further experiments will appear by the writers in the near future.

Experiment.

Moisture: Moisture was determined by K. Kafuku's apparatus. In some cases, it was determined by drying the material to a constant weight at 105°C. The difference in results from both methods was negligible.

Nitrogen: The total nitrogen was determined by Kjeldal's method, and the percentage of the lipoid nitrogen was calculated by determining the nitrogen content of the defatted material.

Ether Extract: The extraction of fatty materials with ether was made with Soxlet's apparatus. An extraction made in 1930 was made from the sample dried at 105°C (A). In all other experiments, oils were extracted from fresh materials after dehydrating with sodium sulphate. As is shown in Table V, the dessication of materials at 105°C seems to affect the acid values of the extract a little.

In the extraction, ethyl ether was used generally as solvent but in two experiments (C & E) petroleum ether (boiling under 60°C) was used. The oily matter extracted with petroleum ether shows a lower iodine value than the usual matter.

Bromination of Lipoid Fatty Acid from Oils of the Larvae (B):

2,2025 gm. acid gave 0,2424 gm. ether insoluble bromide (11%). It melts at 210° with decomposition, and is slightly soluble in chloroform and benzene. The bromine content was determined by micro-Carius' method with the following results:

Sample	Ag Br	Br (found)	Br (calculated)
5.715 mg	8.639 mg	64.33%	as $C_{18}H_{30}O_2Br_6$ 63.3
7.860	11.826	64.05	as $C_{22}H_{34}O_2Br_{10}$ 70.8
7.272	10.894	63.73	
		Mean: 64.04	

When the bromide is mixed with linolenic acid hexabromide (prepared from soy-bean oil, mp. 178°), the mixture melts at 176°. The mixture with clupanodonic acid decabromide (from sardine oil, does not melt at 250°) blackens at 250°, but does not melt.

The bromide is sparingly soluble in benzene. If a large quantity of the solvent is used, we can get crystal with mp. 178–9°.

The molecular weight was determined after Rast.

Sample 1.510 mg.	Camphor 14.962	△6.°0	M.W. 672	M.W. calculated 758 as $C_{18}H_{30}O_2Br_6$
1.186	(mp. 175° △=40) 10.990	5.°5	785	1134 as $C_{22}H_{34}O_2Br_{10}$

Oxidation of Liquid Fatty Acid (after Hazura):

2.7 gm. fatty acid above described were oxidised with 200 cc. 0.5% KOH and 200 cc. 0.5% $KMnO_4$ for 1.5 hours, and 1.5 gm. of unchanged oil which were recovered and were again treated with alkaline $KMnO_4$ solution for 1 hour.

The first oxidation product was acidified to separate free acids which were divided by recrystallisation from boiling water into the following three fractions:

1: m.p. 206°.....Linusic acid?

2: 129–130°, shrinks at 126 (Dioxystearic acid, 133–4°) N.V. 172.3, 168.3, 175.5 (Mean 172; Dioxystearic acid, 177.6).

3: 173°.....Sativic acid.

The second oxidation product was almost composed of dioxystearic acid only.

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ÜBER DEN EINFLUSS DER GALLENSÄURE AUF DIE NUCLEINVERDAUUNG.

1. Bei Verdauung der Nucleinsäure durch Darmsaft.

Von

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(Eingegangen am 31. März 1932)

Bekanntlich handelt es sich bei der Gallensäure um einen enterohepatischen Kreislauf, indem die in den Darm ergossene Gallensäure zum grössten Teil unverändert von der Darmschleimhaut wieder aufgenommen, von der Leber abgefangen und dann aufs neue zur Gallenbildung verwendet wird.

So muss die Verdauung der Nahrung durch die Gallensäure stark beeinflusst werden, und es ist allgemein bekannt, dass die Gallensäure die Verdauung und die Resorption des Fettes im Darm günstig beeinflusst.

Bezüglich des Einflusses der Gallensäure auf die Eiweissverdauung wurde von Karasawa und Shoda (1927) experimentell bewiesen, dass die Gallensäure die Eiweissverdauung durch Trypsin sowie Erepsin hemmt.

Nach der Untersuchung von Levene und seinen Mitarbeitern (1911/13) soll die Nucleinsäure unter der Wirkung eines Nucleotidase genannten Fermentes in Phosphorsäure und Nucleosid zerfallen.

Diese Nucleotidase liess sich im Darmsaft und im Extrakt von Organen, wie Darmschleimhaut, Leber und Pankreas, nachweisen, jedoch übt der Darmsaft des Hundes auf das Nucleosid keinen Einfluss aus.

Über den Einfluss der Gallensäure auf die Nucleinsäurespaltung durch Darmsaft findet sich meines Wissens in der Literatur nichts vor.

Neuerdings wurde der Einfluss der Gallensäure auf die Nucleotidase in Leber und Darm von Okamura (1928/30) untersucht, und gefunden, dass die Spaltung der Nucleinsäure durch den Extrakt von Leber und Darmschleimhaut durch die Gallensäure gefördert wird, indem sich die aus der Nucleinsäure frei gewordene Phosphorsäure dabei vermehrt.

Ich habe den Einfluss der Gallensäure auf die Spaltung der Nucleinsäure durch den Darmsaft des Hundes untersucht.

Experimenteller Teil.

Zwecks Sammlung des Darmsaftes vom Hunde wurde nach der Methode von Bickel 20 cm unterhalb des Pylorus eine Darmfistel angelegt. Nachdem die Operationswunde geheilt war, wurde der Hund zum Versuche verwendet.

Jeden Morgen 8 Uhr wurde der Hund mit einer bestimmten Nahrung gefüttert, und von 9 bis 10 Uhr der Darmsaft aus der Darmfistel in einem Kolben gesammelt. Hierbei wurde der Hund auf dem Gestell ruhig gehalten. Die Nahrung bestand, wie in der Tabelle angegeben ist, aus Reis, Fleisch, Sojasuppe und Wasser.

Der gesammelte Darmsaft wurde zentrifugiert, und der obere klare Teil des Zentrifugates zum Versuch gebraucht.

Als Substrat wurde 2%ige Natriumhefenucleinatlösung verwendet.

Als Puffer fand das Sörensensche Phosphatgemisch von PH=8.043 Verwendung. Es wurden Cholsäure, Glykodesoxycholsäure und Taurocholsäure als Natriumsalz in Lösung verwendet.

Die Versuchsanordnungen sind in den Tabellen I, V und IX zusammengestellt. Das Digestionsgemisch wurde unter Toluol im Brutschrank bei 37°C 24 Stunden lang stehen gelassen.

Als Kontrolle wurde ein Gemisch ohne Gallensäure gebraucht.

Die Gesamtmenge des Gemisches betrug 50 ccm. Bei diesem Gemisch wurde die Spaltung der Nucleinsäure mittelst des Pighinischen optischen Verfahrens (1910) untersucht, und die vom Nucleotid abgespaltete anorganische Phosphorsäure nach der Methode

von Embden gravimetrisch bestimmt. Beim optischen Verfahren wurden 30 ccm Digestionsgemisch unter Zusatz von Tierkohle 25 Minuten lang erwärmt, mit Kaolin umgerührt und abfiltriert. Das klare Filtrat wurde in einem 2 dm langen Beobachtungsrohr optisch untersucht. Da die Gallensäure nach rechts dreht, wurde von dem gefundenen Wert der Drehung der in den Tabellen II, VI und X angegebene der Gallensäure abgezogen.

Bei Feststellung der Drehung der Gallensäure war in der Gesamtmenge der Flüssigkeit ohne Nucleinsäure dieselbe Menge der Gallensäure wie in den Versuchen enthalten. 20 ccm Digestionsgemisch wurden nach Schenck enteiweisst, und die von Eiweiss befreite Flüssigkeit sofort zur Bestimmung der Phosphorsäure verwendet.

Die Resultate sind in den folgenden Tabellen III, IV, VII, VIII, und XI zusammengestellt.

1. Versuch mit Cholsäure.

Die Versuchsanordnungen sind in Tabelle I angegeben; das Gemisch wurde so hergestellt, dass es 0,01–0,03% Gallensäure enthielt, indem verschiedene Mengen einer 0,1–1,0%igen Cholsäurelösung dem Gemisch zugesetzt wurden.

Aus den Tabellen III und IV geht hervor, dass bei einem 0,01–0,05%igen Gehalt an Cholsäure im Digestionsgemisch die Spaltung der Nucleinsäure durch Darmsaft gefördert wurde, was daraus zu ersehen ist, dass der Drehungsgrad des Gemisches dem der Kontrolle gegenüber (Nr. 1) abgenommen, und die dabei gespaltete Phosphorsäure im Vergleich damit zugenommen hatte.

Bei einem 0,1%igen Cholsäuregehalt wurde aber die Nucleinsäurespaltung durch Darmsaft in der Mehrzahl der Fälle gehemmt, obwohl bei einigen Fällen, verglichen mit der Kontrolle (Nr. 1), Abnahme der optischen Drehung und Zunahme der gespalteten Phosphorsäure beobachtet wurden.

Bei einem 0,2 oder 0.3%igen Cholsäuregehalt (Nr. 6 und 7) wurde die Nucleinsäurespaltung durch Darmsaft ohne Ausnahme in allen Fällen deutlich herabgesetzt.

Tabelle I.

Bestandteile des Digestionsgemisches: Nr. der Kölbchen / ccm	1	2	3	4	5	6	7
Darmsaft	5	5	5	0	5	5	5
2%ige Na-Nucleinatlös.	20	20	20	20	20	20	20
Phosphatgemisch	10	10	10	10	10	10	10
Physiologische NaCl-Lös.	15	10	10	15	10	5	0
1%ige Na-Cholatlös.	0	0	0	5	5	10	15
0,5%ige Na-Cholatlös.	0	0	5	0	0	0	0
0,1%ige Na-Cholatlös.	0	5	0	0	0	0	0
Toluol	0,5	0,5	0,5	0,5	0,5	0,5	0,5
Menge des Gemisches in ccm	50	50	50	50	50	50	50
Cholsäuregehalt des Gemisches in %	0	0,01%	0,05%	0,1%	0,1%	0,2%	0,3%

Also hängt die die Nucleinsäurespaltung durch Darmsaft fördernde Wirkung der Cholsäure von der Menge derselben ab.

Tabelle II.

	Drehungsgrad bei 23°C
0,01%ige Na-Cholatlösung	0,02
0,05%ige Na-Cholatlösung	0,05
0,1 %ige Na-Cholatlösung	0,10
0,2 %ige Na-Cholatlösung	0,20
0,3 %ige Na-Cholatlösung	0,30

Tabelle III.

Versuch mit dem Darmsaft von Hund A.

(Nahrung: Reis 250 g, Fleisch 150 g, Sojasuppe 20 ccm und Wasser 1000 ccm)

Datum des Versuches	Nr. der Kölbchen / gefundene Werte	1	2	3	4	5	6
25/V	Drehungsgrad (Temperatur)	1,02 (23°)	1,00 (〃)	0,99 (〃)	1,85 (〃)	1,10 (〃)	1,13 (〃)
	P_2O_5 in mg	51,59	51,97	52,12	50,48	51,63	51,44
1/VI	Drehungsgrad (Temperatur)	0,94 (23°)	0,92 (〃)	0.90 (〃)	1,86 (〃)	0,93 (〃)	0,95 (〃)
	P_2O_5 in mg	59,38	60,87	61,73	51,44	58,32	58,13
5/VI	Drehungsgrad (Temperatur)	1,10 (23°)	1,09 (〃)	1,04 (〃)	2,08 (〃)	1,10 (〃)	1,13 (〃)
	P_2O_5 in mg	58,37	58,46	58.80	52,36	57,88	56,92

Tabelle IV.

Versuch mit dem Darmsaft von Hund B.

(Nahrung: Reis 200 g, Fleisch 150 g, Sojasuppe 20 ccm und Wasser 800 ccm)

Datum des Versuches	Nr. der Kölbchen / gefundene Werte	1	2	3	4	5	6	7
8/VI	Drehungsgrad (Temperatur)	0,99 (23°)	0,98 (〃)	0,97 (〃)	1,82 (〃)	1,00 (〃)	1,03 (〃)	1,08 (〃)
	P_2O_5 in mg	64,77	64,90	65,05	53,99	64,04	62,55	61,15
18/VI	Drehungsgrad (Temperatur)	1,41 (23°)	1,39 (〃)	1,37 (〃)	2,20 (〃)	1,38 (〃)	1,42 (〃)	1,45 (〃)
	P_2O_5 in mg	57,93	58,17	58,65	51,63	58,37	57,69	57,45
21/VI	Drehungsgrad (Temperatur)	1,57 (23°)	1,55 (〃)	1,54 (〃)	2,17 (〃)	1,56 (〃)	1,60 (〃)	
	P_2O_5 in mg	55,53	55,97	56,30	52,07	55,96	55,14	

2. Versuch mit Glykodesoxycholsäure.

Die Glykodesoxycholsäure gewann Fuziwara aus Kaninchengalle. Ich möchte ihm an dieser Stelle für freundliche Überlassung derselben meinen herzlichen Dank aussprechen. Diese Säure wurde als 0,01–0,2%ige Natriumsalzlösung zum Versuch verwendet. Die Versuchsanordnungen sind in Tabelle V angegeben.

Nach den Tabellen VII und VIII wird die Spaltung der Nucleinsäure durch Darmsaft bei 0,01%igem Gehalt an Glykodesoxycholsäure im Vergleich mit der Kontrolle (Nr. 1) gefördert, während sie bei 0,05%igem (Nr. 3) sehr schwach, aber bei 0,1–0,2%igem (Nr. 5 und 6) deutlich gehemmt wird.

Diese die Nucleinsäurespaltung durch Darmsaft hemmende Wirkung der Glykodesoxycholsäure tritt viel stärker auf als die der Cholsäure.

Tabelle V.

Bestandteile des Digestionsgemisches	Nr. der Kölbchen / ccm	1	2	3	4	5	6
	Darmsaft.	5	5	5	0	5	5
	2%ige Na-Nucleinatlös.	20	20	20	20	20	20
	Phosphatgemisch.	10	10	10	10	10	10
	Physiologische NaCl-Lös.	15	10	10	15	10	5
	1%ige Na-Glykodesoxycholatlös.	0	0	0	5	5	10
	0,5%ige Na-Glykodesoxycholatlös.	0	0	5	0	0	0
	0,1%ige Na-Glykodesoxycholatlös.	0	5	0	0	0	0
	Toluol	0,5	0,5	0,5	0,5	0,5	0,5
Menge des Gemisches in ccm		50	50	50	50	50	50
Glykodesoxycholsäuregehalt des Gemisches in %		0	0,01%	0,05%	0,1%	0,1%	0,2%

Tabelle VI.

	Drehungsgrad bei 23,5°C
0,01%ige Na-Glykodesoxycholatlösung	0,05
0,05%ige Na-Glykodesoxycholatlösung	0,10
0,1 %ige Na-Glykodesoxycholatlösung	0,15
0,2 %ige Na-Glykodesoxycholatlösung	0,25

Tabelle VII.

Versuch mit dem Darmsaft von Hund A.

(Nahrung: Reis 250 g, Fleisch 150 g, Sojasuppe 20 ccm und Wasser 1000 ccm)

Datum des Versuches	Nr. der Kölbchen / gefundene Werte	1	2	3	4	5	6
1/VII	Drehungsgrad (Temperatur)	1,04 (23,5°)	1,00 (〃)	1,20 (〃)	2,07 (〃)	1,23 (〃)	1,30 (〃)
	P_2O_5 in mg	56,54	57,12	56,01	53,85	55,77	55,29
5/VII	Drehungsgrad (Temperatur)	1,15 (23,5°)	1,12 (〃)	1,33 (〃)	2,00 (〃)	1,35 (〃)	1,38 (〃)
	P_2O_5 in mg	55,63	53,20	55,58	52,26	55,34	53,13

Tabelle VIII.

Versuch mit dem Darmsaft von Hund B.

(Nahrung: Reis 250 g, Fleisch 150 g, Sojasuppe 20 ccm und Wasser 800 ccm)

Datum des Versuches	Nr. der Kölbchen / gefundene Werte	1	2	3	4	5	6
10/VII	Drehungsgrad (Temperatur)	1,20 (23,5°)	1,25 (〃)	1,50 (〃)	2,13 (〃)	1,52 (〃)	1,55 (〃)
	P_2O_5 in mg	58,80	58,65	57,45	52,12	57,07	54.76
13/VII	Drehungsgrad (Temperatur)	1,22 (23,5°)	1,18 (〃)	1,50 (〃)	1,95 (〃)	1,52 (〃)	1,55 (〃)
	P_2O_5 in mg	59,71	61,15	59,18	56,97	58,89	58,03

3. Versuch mit Taurocholsäure.

Bei diesem Versuch wurde, wie in Tabelle IX angegeben ist, eine 0,1–0,2%ige Natriumtaurocholatlösung von Merk im Verhältnisse von 0,01–0,5% Natriumtaurocholat zum Digestionsgemisch zugesetzt.

Nach Tabelle XI wurde die Spaltung der Nucleinsäure durch Darmsaft bei einem 0,01–0,3%igen Gehalt an Taurocholsäure im

Digestionsgemisch (Nr. 2–7) gefördert, und diese Förderung trat fast parallel mit der Menge der Taurocholsäure auf.

Erst bei einem 0,4–0,5%igem Gehalt an Taurocholsäure wurde die Nucleinsäurespaltung durch den Darmsaft gehemmt. Aus dem allem geht hervor, dass die die Nucleinsäurespaltung durch Darmsaft hemmende Wirkung der Taurocholsäure erst bei grösserer Konzentration dieser Säure auftritt.

Die Gallensäure fördert also die Wirkung der Nucleotidase im Darmsaft, und diese Förderung tritt bei Gegenwart von Taurocholsäure am stärksten auf. Schwächer ist die Förderung bei Cholsäure und am schwächsten bei Glykodesoxycholsäure. Wie Karasawa(1926) und Hatakeyama(1928) bereits nachgewiesen haben, wird der Nucleinstoffwechsel eines Organs durch Zufuhr von Gallensäure stets gefördert, während die Nucleinsäurespaltung durch Darmsaft bei Zusatz von grösseren Mengen von Gallensäure gehemmt wird, wobei die für diese Hemmung notwendige Grenzkonzentration je nach der Art der Gallensäure ganz verschieden ist. Dieser Unterschied der Gallensäurewirkung auf die Nucleinsäurespaltung im Darmsaft und in den Organen scheint mir entweder von der Verschiedenheit der Nucleotidasen oder von der des Wirkungsmechanismus der Gallensäure bei der Verdauung und dem Stoffwechsel abzuhängen, besonders wohl von der letzteren Verschiedenheit.

Die im Vergleich mit der Cholsäure stärker fördernde Eigenschaft der Taurocholsäure hinsichtlich der Nucleinsäurespaltung ist der Entgiftung der Cholsäure zuzuschreiben, indem diese sich mit Taurin paart.

Die die Nucleinsäurespaltung am stärksten hemmende Wirkung der Glykodesoxycholsäure dürfte wohl von der Desoxycholsäure herrühren, an die im Molekül der Glykodesoxycholsäure die stärkste Wirkung gebunden ist, indem die Desoxycholsäure stärker haemolytisch als die Cholsäure wirkt, obwohl sich die Desoxycholsäure bei ihrer Entgiftung mit Glykokoll paart.

Tabelle IX.

Bestandteile des Digestionsgemisches	Nr. der Kölbchen / ccm	1	2	3	4	5	6	7	8	9
	Darmsaft.	5	5	5	0	5	5	5	5	5
	2%ige Na-Nucleinat-Lös.	20	20	20	20	20	20	20	20	20
	Phosphatgemisch.	10	10	10	10	10	10	10	10	10
	Physiologische NaCl-Lös.	15	10	10	15	10	10	5	5	0
	2%ige Na-Taurocholat-Lös.	0	0	0	0	0	5	5	10	10
	1%ige Na-Taurocholatlös.	0	0	0	5	5	0	5	0	5
	0,5%ige Na-Taurocholatlös.	0	0	5	0	0	0	0	0	0
	0,1%ige Na-Taurocholatlös.	0	5	0	0	0	0	0	0	0
	Toluol	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5
Menge des Gemisches in ccm		50	50	50	50	50	50	50	50	50
Taurocholsäuregehalt des Gemisches in %		0	0,01%	0,05%	0,1%	0,1%	0,2%	0,3%	0,4%	0,5%

Tabelle X.

	Drehungsgrad bei 30°C
0,01 %ige Na-Taurocholatlösung	0,02
0,05 %ige Na-Taurocholatlösung	0,06
0,1 %ige Na-Taurocholatlösung	0,08
0,2 %ige Na-Taurocholatlösung	0,13
0,3 %ige Na-Taurocholatlösung	0,18
0,4 %ige Na-Taurocholatlösung	0,21
0,5 %ige Na-Taurocholatlösung	0,26

Tabelle XI.

Versuch mit dem Darmsaft von Hund B.

(Nahrung: Reis 200g, Fleisch 150g, Sojasuppe 200ccm und Wasser 800ccm)

Datum des Versuches	Nr. der Kölbchen / gefunden: Wert:	1	2	3	4	5	6	7	8	9
25/VIII	Drehungsgrad (Temperatur)	1,74 (30°)	1,71 (〃)	1,64 (〃)	1,68 (〃)	1,57 (〃)	1,47 (〃)			
	P_2O_5 in mg	54,09	54,71	54,81	52,55	54,90	55,87			
31/VIII	Drehungsgrad (Temperatur)	1,45 (30°)		1,39 (〃)	2,07 (〃)	1,35 (〃)	1,38 (〃)	1,40 (〃)	1,49 (〃)	1,56 (〃)
	P_2O_5 in mg	55,63		56,44	54,13	57,31	56,54	56,25	54,76	53,61

Zusammenfassung.

1. Die Nucleinsäurespaltung durch den Darmsaft des Hundes wird durch Gallensäure in schwächerer Konzentration gefördert, aber in stärkerer gehemmt.

2. Diese förderende und hemmende Wirkung der Gallensäure ist je nach der Art der Gallensäure ganz verschieden. Am stärk-

sten fördert die Taurocholsäure, weniger stark die Cholsäure und am schwächsten die Glykodesoxycholsäure.

Aus meinen Ergebnissen geht hervor, dass die Verdauung der Nucleinsäure im Darm durch Gallensäure von bestimmter Konzentration gefördert wird.

Zum Schluss möchte ich Herrn Prof. Dr. T. Shimizu für seine freundliche Anleitung bei meiner Arbeit herzlichst danken.

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THE INFLUENCE OF BILE ACID UPON THE MUTAROTATION OF GLUCOSE.

By

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In 1927 Misaki reported that the level of blood sugar of normal, hyperglycemical and adrenaline-hyperglycemical rabbit is taken down by the administration of bile acid, and that the secretion of adrenaline is suppressed by the bile acid, and that consequently the content of glycogen in the liver and muscle increases. Murakami (1928) showed that the hyperglycemia produced by the administration of glucose is checked by the administration of bile acid. Okamura (1928) pointed out that as a result of the loss of bile acid out of the body the amount of the sugar in the blood increases, and when the acid is administered the level of sugar falls again. Recently Fuzita (1930) demonstrated that the content of glycogen of liver increases by the administration of bile acid. In short, the bile acid takes part in the carbohydrate metabolism of an animal and accelerates the process of building glycogen from sugar. I worked with a view to making clear this chemism by polarimetric observations.

Biot (1818) found the optical activity of glucose. Dubrunfaut (1846) reported that the freshly prepared aqueous solution of glucose gives birotation, and the ability of rotation goes down in course of time, but this reaction is accelerated by heating and addition of acid, and when the solution is boiled, the rotation is reduced instantly to the constant value. Tollens und Schulze (1892) studied deeply into the problem and noted that the mutarotation of sugar is reduced to normal and subnormal values when the sugar is dissolved in a concentrated ammonia. Osaka (cited by Bleyer and Schmidt) studied the relationship between

the mutarotation and the concentration of hydrogen ions, and found the general rule that the speed-constant of reaction is proportional to the concentration of OH-ions and the mutarotation is stopped instantly by the addition of weak ammonia; and on the contrary, H-ion exercises less influence upon the mutarotation, and the speed of the reaction is proportional to the square root of the concentration of H-ions. But the result of Murschhauser's (1922) experiment was somewhat different from this rule.

Many theories were established for the explanation of the mutarotation of sugar. Biot (cited by Bleyer and Schmidt) explained that the fall of beginning rotation is due to the decomposition of large complex of glucose molecules in course of time. Hammerschmidt observed that the mutarotation is a physiological and chemical phenomenon, and it occurs at a result of the appearing of glucose molecules at the same time and yields the molecular complex which is krystallinically and optically active. Whether these theories are right or not can easily be proved by the measurement of the freezing point of the solution of glucose by the Raoult's method; that is, some difference must exist between the molecular weights of glucose in the solution prepared fresh and the one left standing for a long time. However, Arrhenius' experiments showed that this theory was wrong, so it ended only as a hypothesis without experimental proof. E. Fischer (1890), Tollens (1893), Bechámp (1893) established the so-called hydrate theory. According to this theory, mutarotation is due to the addition of water molecules to the glucose and to the splitting off from it. E. Fischer stated that when the strongly rotating anhydride of glucose was dissolved in the water, the water molecules were combined to the anhydride and became hydrate which rotated lower by and by. On the contrary, Tollens and Bechámp observed that hydrate becomes anhydride by splitting off the water molecules.

Edmund von Lippmann was the first who gave a satisfactory explanation of the mutarotation of sugar. He said that the mutarotation is due to the fact that a high and a low rotating stereoisomeric form exist at the same time, and in free state

glucose binds like ethylenoxyde, and so the asymmetry of the fifth C-atom represents different rotations. For this theory, experimental supports were made by Erdmann (1855), Schmöger (1880–1881), E. Fischer (1890), Behrend and Roth (1904–1907) and Hudson (1903–1909).

In short, mutarotation occurs in consequence of the transformation from one form into another isomeric form, and the constant rotation is one that takes place when the two forms exist in a state of equilibrium.

Hudson (1909) studied the mutarotation of aldose, and found that aldose has two isomeric forms, each of them having its own specific rotation, and that when sugar is dissolved in water, one form is transformed into the other, and that the terminal C-atom of aldehydegroup of these two forms binds with γ-carbonatom as lactone, so the terminal C-atom is asymmetric; so that the two modifications (α- and β-form) are obtained by the arrangement of atoms which are combined with the C-atom. Further, Bleyer and Schmidt (1923) studied the effect of alkali and acid on the mutarotation of sugar and concluded that it was certain that hexose and disaccharide change into compounds with less carbon atom by the effect of OH-ions, especially on the glucose, and he stated that β-form is transformed into a carbon ring under isometrisation which is decomposed into a glycerinaldehyde getting two molecules of water owing to its lability. Hewitt and Pryde (1920) commented that the most outstanding feature of this newly described type of sugar γ-glucose is the speed and range it undergoes. For example, condensation with an alcohol to form the corresponding glucoside takes place in a few minutes and the similar reaction with acetone is practically instantaneous. By comparison, the normal forms of reducing sugars are relatively stable, inert substances, and the possibility thus suggests itself that in the rapid transformations of carbohydrate in living tissues, γ-sugars may play an important, if not essential, part in both animal and vegetable metabolic processes.

Winter and Smith (1922) found from their experiments that blood sugar exists as a form of so-called γ-glucose. The

glycogen is polymerized from monosaccharides (glucose, fructose etc.) which are freed from disaccharide and polysaccharide under the action of ferment in the intestine. Now, the bile acid is built from its mother substances in the liver and some portion is removed out of the body in the faeces, some portion is decomposed, and the greater part of the rest is absorbed by the intestine and builds bile. So it is to be inferred that the bile acid comes into contact with sugar in the intestine, blood and liver. There are three ways in which the bile acid takes part in the building of the glycogen in the liver. First, the bile acid influences the sugar directly and helps to transform the d-glucose into reaction form. Secondly, the bile acid changes the reaction of the medium of the sugar, and causes glycogen to be easily built. Lastly the bile acid activates the ferment in the liver and helps to build glycoken from sugar.

The first hypothesis was proved by Hatakeyama (1928) experimentally, and my experimental results coincide with it. The change in the specific rotation of glucose is accelerated by the addition of sodiumcholate. The second hypothesis is supported by the works of Karasawa (1926–1927), Hatakeyama (1927), Takezi Okamura (1928–1930), Sekitoo (1929), Fuziwara (1931) and Hosizima (1931). When the amount of bile acid contained in the blood is increased by the injection of it, or as a result of congestional jaundice, the amount of phosphate in the blood and urine increases; and especially, as Fuziwara proved, the content of secondary phosphate in the urine increases, because of the acceleration of bile acid on the nuclease. From these works it can be inferred easily that the buffer-content and the OH-ion-concentration and at least the alkali reserve increase in the blood.

I plan to make clear the effect of the bile acid on the speed of the fall of specific rotation of glucose and the final value of it by the change of pH and amount of buffer, and further show how the bile acid influences the formation of glycogen in the liver.

Experiment.

As the sugar, E. Merk's purest glucose was chosen, which was used as 0.5 per cent solution. The reaction of cholate is of course

exactly neutral. The ferment solution was prepared as follows: the liver which was freshly taken from a rabbit was ground into the form of gruel, and ten times the amount of chloroform-water was given, and after shaking it completely it was kept for four days in the incubator at 37°C for autolysis, and then was slightly acidified with acetic acid, left overnight, and filtered. The filtrate was dialised in the collodiumsack and filtered again. The enzyme solution obtained had a tint of orange, but was entirely clear; reacted neutral and had no optical activity. As buffer, Sörensen's phosphate of 1/15 mol solution was applied. The polarimeter used in this work was Schmidt and Haensch's half shadow apparatus. The length of the observation tube was 2 dm; the wave length of polarized light was 535 $\mu\mu$.

I. The effect of the change of pH and the amount of buffer on the specific rotation of glucose.

Table I.

A.	pH 7.38	A_1, B_1, C_1.	buffer 5 cc.
B.	pH 8.04	A_2, B_2, C_2.	buffer 10 cc.
C.	pH 8.68	A_3, B_3, C_3.	buffer 20 cc.
		D.	buffer 0

Min.	A_1	A_2	A_3	B_1	B_2	B_3	C_1	C_2	C_3	D
0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
5	0.94	0.91	0.85	0.93	0.90	0.84	0.93	0.90	0.84	0.98
15	0.85	0.81	0.70	0.83	0.78	0.69	0.82	0.76	0.69	0.94
30	0.75	0.70	0.63	0.73	0.66	0.62	0.72	0.65	0.61	0.90
45	0.68	0.65	0.60	0.67	0.63	0.59	0.65	0.63	0.60	0.87
60	0.64	0.63	0.60	0.62	9.60	0.59	0.62	0.60	0.61	0.83
120	0.60	0.60	0.60	0.59	0.60	0.60	0.60	0.60	0.60	0.72
180	0.60	0.60	0.60	0.60	0.60	0.60	0.59	0.60	0.61	0.67
240										0.64
360										0.61
430										0.60
1080										0.60

In these experiments, observations were begun when the reading of the rotation reached about 1.00 as is shown in the table.

Comparison between A and D: in case A, the rotation was reduced to the constant value in 120 minutes; on the other hand, in case D which had no buffer, the rotation was reduced to the constant value in 480 minutes.

It is plain that the alkalinity of the medium of glucose accelerated the change in its specific rotation. This result coincides with many other workers' reports. Of course, the time to reach 1.00 of the reading of the rotation was longer in case D than in A, though it was not measured exactly. When A_1, A_2, A_3; B_1, B_2, B_3; C_1, C_2, C_3, are compared, you will find that the time taken to reduce to constant rotation is short as the amount of buffer increases. And if you compare A_1, B_1, C_1; A_2, B_2, C_2; A_3, B_3, C_3, you can observe that the increase of alkalinity and the acceleration of the reaction to transform glucose in stereoisomeric form which has minor specific rotation is roughly parallel.

II. The activity of liver ferment on the mutarotation of glucose and the effect of bile acid on it.

Table II (A)

Observation temperature 37°C.
A ..... glucose, buffer.
B ..... glucose, buffer, solution of ferment.
C ..... glucose, buffer, solution of ferment, sodiumcholate.
D ..... glucose, buffer, sodiumcholate.
E ..... sodiumcholate.
F ..... buffer.
G ..... solution of ferment.
pH..... 7.38.

Min.	A	B_1	B_2	B_3	C_1	C_2	C_3	D
5	1.03	0.92	0.95	0.94	0.96	0.97	0.97	1.01
10	0.81	0.71	0.78	0.76	0.79	0.79	0.79	0.78
15	0.72	0.67	0.71	0.69	0.73	0.72	0.71	0.72
20	0.70	0.65	0.70	0.68	0.71	0.71	0.70	0.68
30	0.69	0.65	0.68	0.67	0.70	0.68	0.68	0.67
45	0.69	0.64	0.68	0.67	0.70	0.68	0.68	0.67
60	0.69	0.65	0.68	0.67	0.70	0.69	0.68	0.67

Table II (B)

Min.	E	F	$G_{1,2,3}$	C_1–E	C_2–E	C_3–E	D–E
5	0.08	0	0	0.88	0.89	0.89	0.93
10	„	„	„	0.71	0.71	0.71	0.70
15	„	„	„	0.65	0.68	0.63	0.64
20	„	„	„	0.63	0.63	0.62	0.60
30	„	„	„	0.62	0.60	0.60	0.59
45	„	„	„	0.62	0.60	0.60	0.59
60	„	„	„	0.62	0.60	0.60	0.59

The ferment solution of liver (G) was prepared from three sources. The number 1, 2, 3 denotes that of the experiment. The buffer and the solution of ferment had no optical activity. In these experiments, the first observations were done exactly five minutes after the solution was prepared.

A compared with B: in each case the rotation was reduced to the constant value in thirty minutes, but in case B, the fall of rotation was faster than of that in A; and the final rotation in A was higher than that in B. So it can be inferred that some fermentation occurred in B. A compared with D–E: the initial rotation in A was higher than that in D-E, and both of them became constant in thirty minutes. And the final rotation was A>D-E. From this fact it is clear that cholate accelerates the transformation of glucose in isomeric form, which coincides with Hatakeyama's work. Now compare B with C-E, and you will find that the fall of the rotation of the former is faster than that of the latter, and the final rotation is B>C-E, that is, the ferment of the liver is activated by the sodiumcholate.

In this case the pH 8.04 was chosen instead of 7,38, the experimental results of which were described in table 2, and they were the same as that of pH 7.38.

Conclution.

From my experiments above mentioned, it can be concluded that there are three ways in which the bile acid degrades the level

Table III (A)

Observation temperature 37°C.
A ..... glucose, buffer.
B ..... glucose, buffer, solution of ferment.
C ..... glucose, buffer, solution of ferment, sodiumcholate.
D ..... glucose, buffer, sodiumcholate.
E ..... sodiumcholate.
F ..... buffer.
G ..... solution of ferment.
pH..... 8.04.

Min.	A	B_1	B_2	B_3	C_1	C_2	C_3	D
5	1.02	0.91	0.94	0.92	0.96	0.95	0.95	1.00
10	0.81	0.70	0.77	0.75	0.78	0.78	0.77	0.80
15	0.71	0.65	0.71	0.67	0.73	0.71	0.70	0.74
20	0.70	0.65	0.69	0.66	0.70	0.70	0.67	0.72
30	0.69	0.65	0.67	0.66	0.70	0.69	0.67	0.70
45	0.70	0.65	0.67	0.66	0.70	0.69	0.66	0.70
60	0.70	0.65	0.68	0.67	0.70	0.69	0.67	0.70

Table III (B)

Min,	E	F	$G_{1,2,3}$	C_1–E	C_2–E	C_3–E	D–E
5	0.08	0	0	0.88	0.87	0.88	0.92
10	„	„	„	0.70	0.70	0.69	0.72
15	„	„	„	0.65	0.63	0.62	0.66
20	„	„	„	0.62	0.62	0.59	0.64
30	„	„	„	0.62	0.61	0.59	0.62
45	„	„	„	0.62	0.61	0.59	0.62
60	„	„	„	0.62	0.61	0.59	0.62

of blood sugar and increases the content of glycogen in the liver: 1) the increase of alkalinity, 2) the increase of the content of phosphorous in the blood, 3) the direct action of bile acid upon the sugar in the blood and the ferment in the liver.

It seems that all these agencies combine to accelerate the transformation of the common glucose into a reaction form.

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THE ACTION OF SUGAR ON AMINO ACID.

I. The Reaction in Alkaline Medium.

By

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The chemical mechanism by which the reactions of amino acids with sugars are accomplished has long been the subject of investigation in the fields of biological, industrial and pure chemistry. These reactions seem to take place readily even under the mildest conditions. Neuberg and Kobel (1925; 1926 i) noticed the instantaneous change of optical rotation when the solutions of amino acids were mixed with sugars, particularly with fructose or hexose-di-phosphate at neutral reaction and room temperature. It was found by the same investigators (1926 ii; 1927 i) that the protein and its decomposition-products reacted also promptly with fructose and hexose-mono-phosphoric esters. This view was strongly substantiated by a series of experiments carried out by Euler and his associates (1926–1927). They found that the reactions between amino acids and sugars, which were reversible, took place at equimolecular ratio, and that their velocity became greater in higher alkaline medium. Pringsheim and Winter (1926; 1927) concluded, from the regular decrease of sugars in the mixed solutions of sugars and proteins, that the latter condensed momentarily with the former, and that the condensation resulted in the production of stable compounds. The weight of evidence is, however, against this view. Thus Sörensen and Lorber (1927), Euler and Brunius (1927), and Neuberg and Simon (1927) pointed out Pringsheim and Winter's conclusion to be erroneous. In their study on the action of enzyme Waldschmidt-Leitz and Rauchalles (1928) observed that

the optimum hydrogen-ion concentration for the velocity of the reactions between dipeptides and glucose was pH 8. The fact that the velocity of diffusion of the mixed watery solutions of amino acids and glucose did not differ from that of the pure solution of glucose, led Krüger (1929) to the assumption that the reaction might take place between glucose and amino acids only in the alkaline medium.

The facts so far brought forward afford no definite chemical proof for the reaction between amino acid and sugar, since the reaction is of an extraordinarily reversible nature and takes place without the formation of any definite reaction-products. However, more deep-seated changes which present a clue to the nature of the chemistry involved in the reaction of amino acids with sugars have been often studied in industrial chemistry. Most of the theories of these reactions which were commonly induced in the neutral medium by the application of high temperature, postulated as a first step the reaction between the amino group of the acid and the aldehyde group of the sugar. The chief reaction-products were found to be melanoidins, aldehydes and carbon dioxide. The old contributions of Maillard (1912), Lintner (1912), Ruckdeschel (1914) and others support this view. The literature of the last few years has clarified to a large extent the mechanism concerning the reaction. Dowell and Menaul (1919) observed the great decrease of amino-nitrogen of the protein-hydrolysates or amino acids when these substances were brought to react with furfural or dextrose at a neutral or slightly acid medium. Grünhut and Weber (1921) examined extensively the reactions between amino acids and sugars by determining amino-nitrogen, reducing power and optical rotation of the mixtures after they had been repeatedly evaporated and redissolved in water. The reactivity of amino acids towards sugars decreased in the order named: Glutamic acid, glycine, alanine and leucine. Aspartic acid reacted only with arabinose. The order of the reactivity of sugars was as follows: Arabinose, glucose, galactose, fructose, disaccharides and raffinose. The rate of the liberation of carbon dioxide was similar to that of ammonia when glutamic acid was

employed. In the cases of glycine and alanine the deamination took place with greater velocity than the CO_2-liberation when they were brought to contact with monosaccharides, while with disaccharides the decrease of amino-nitrogen proceeded without carrying with it the formation of carbon dioxide. The formation of melanoidins was always noticed in the reaction, with the exception of the case where leucine was used. Borsook and Wasteneys (1925) found that in the presence of glucose the free amino-nitrogen of glycine and of other more complex products of protein-decomposition decreased in the alkaline solution, but never in the acid solution. Ripp (1926) followed chemically and optically the reactions of amino acids and reducing sugars at 100°C. He also examined the nature of melanoidins formed as the result of heating fructose with alanine, asparagine, glutamine, glutamic and aspartic acid. Lundin (1929) considered the reaction between glycocoll and glucose as one of the possible causes of the inhibitory action of the presence of the amino acid on the determination of glucose by the Folin-Wu or Folin reagent. According to Ambler (1929) the course of the reaction between glucose and amino acids depended on temperature, duration of heating, concentration of reacting substances, etc. When volatile products were removed by boiling the solution, the consumption of glucose caused by amino acids was found to be 10 or more mols per mol of amino acids. At a temperature below boiling point a part of the amino acids was destroyed by the side reaction induced by the aldehydes which had been produced in the primary reaction, and thus the molecular proportions of reacting glucose to amino acids were lowered. Acetaldehyde was produced from alanine and asparagine, while glycocoll gave no indication of the formation of formaldehyde. Carbon dioxide was formed only when relatively concentrated solutions were employed. Ambler assumed that glucose might suffer dehydroxylation during the reaction, thus resulting in the production of compounds of progressive complexity and of increasing degrees of unsaturation and polymerization.

The problem concerning the reaction between the amino acid and sugar has been particularly attractive to Japanese chemists,

because of the fact that the aroma and colour of soy originate in the reaction-products of amino acids with sugars. Kodama (1922) isolated, as the aromatic substances of soy, two kinds of unsaturated ketonaldehydes from the fractional distillates of the ether-extract obtained from the steam-distillate of soy. He deduced the molecular formulae of these substances to be $C_5H_6O_2$ and $C_6H_8O_2$. Kurono (1924–1925) and Kurono and Fukai (1928) isolated a ketonaldehyde in the form of aldomedon (mp.=94–95°) from the steam-distillate of soy. From the analytical results they concluded that this substance should be γ-acetobutyraldehyde CH_3-CO-CH_2-CH_2-CH_2-CHO. They named it "soyanal". Soyanal was obtained *in vitro* by fermenting the mixtures of amino acids and methylpentose or pentoses with soy-yeast. The investigators then synthesized the same substance from the mixed glycerol-solutions of amino acids and sugars heated at 130°C. Among the sugars, rhamnose was most effective as the source of soyanal. Next to it came, in the order named, lactose, mannose, galactose, glucose, maltose and sucrose. Xylose and arabinose were found to be least capable of forming soyanal, and fructose and raffinose to be ineffective. When amino acids were arranged according to their effectivity the order was as follows: Leucine, sodium salt of glutamic acid, glycine, asparagine, alanine, tyrosine and phenylalanine. From the fact that the same soyanal was always formed in spite of the varying kinds of amino acids the investigators assumed that the material which occupied the main situation in the chemistry of the soyanal production might be the sugar, particularly methylpentose, and that the amino acid might play a supporting rôle. The colouring substance of soy, "soyamelanin", was also isolated by Kurono from the reaction products.

Akabori (1927; 1931 i) observed the production of aldehydes which contained an atom less of carbon than the corresponding amino acids when amino acids were heated with sugars in the medium of glycerol at 120–130°C. Thus acetaldehyde was produced from alanine, isovaleraldehyde from leucine, isobutyraldehyde from valine, phenylacetaldehyde from phenylalanine and so on. At the same time furfurals corresponding to the sugars

used were always found in a small amount; glucose, arabinose and rhamnose gave rise to the production of oxymethyl-furfural, furfural and methyl-furfural respectively. The liberation of carbon dioxide was always noticed during the reaction. Ammonia was never detected in free form, while the production of brown coloured nitrogenous substances ran parallel with the reaction. He supposed that the ammonia once produced might participate, in the nascent state, in the formation of melanoidins. The mechanism of the oxidation of the amino acid by the sugar was explained by him in the following formula:

$$\begin{matrix} \text{R–CH–COOH} \\ \quad | \\ \quad \text{NH}_2 \end{matrix} + \tfrac{1}{2}\text{O}_2 \rightarrow \text{R–CHO} + \text{CO}_2 + \text{NH}_3$$

In the meantime the same investigator (1931 ii) pointed out the reaction of amino acids with furfurals. The mode of reaction was very much the same as in the case where sugar was used in place of furfural. Akabori's view was fully substantiated by Yamada (1928) who isolated aldehydes and furfurals in the distillates obtained from the mixtures of amino acids and sugars.

The results so far reviewed, taken as a whole, seem to force us to the conclusion that the reaction of the amino acid with sugar takes place at neutral reaction and high temperature between the amino-group of the former and the aldehyde group of the latter, with the production of carbon dioxide, melanoidins, furfurals and aldehydes. The fact that the reaction carries with it the production of nitrogenous melanoidins but not the formation of free ammonia led Akabori to suggest that the amino acid and sugar might first combine to form an unknown compound which might in turn decompose with the production of an aldehyde and carbon dioxide. It should be recalled in this connection that Irvine and Hynd (1911) reported to have synthesized glucose-alanide.

Since the decomposition-products of the sugar also react with the amino acid, the problem of the reaction between the amino acid and sugar may become quite complicated when the sugar which is in the course of progressive disintegration is dealt with. It was pointed out by Neuberg and Kobel (1927 ii & iii) that the amino

acid reacted immediately with methylglyoxal, a decomposition-product of the sugar, with the production of ammonia, carbon dioxide and aldehyde of the next lower acid. Sakuma (1930) found that a molecule of methylglyoxal reacted with a molecule of the mono-amino acid, and that the amount of ammonia and carbon dioxide liberated covered 70 to 80% of the quantity of the amino acid which had disappeared.

The data considered here represent the reactions between amino acids and sugars which were in process of decomposition caused by the action of alkali. It was repeatedly observed at various alkalinities of the medium that sugars accelerated the deamination of amino acids, and that the latter retarded the disintegration of the former.

The Reaction at 0.5N Alkalinity.

The experiments were carried out under anaerobic and CO_2-free conditions, covering the CO_2-free solution with mineral oil. Generally 16.67 cc. of 1*M* amino acids solutions and various quantities of sugars were diluted to 100 cc. with sodium hydroxide solution, the final concentration of which was 0.5*N*, and the mixtures were kept at 37.5°C. This condition was taken as the standard in all of the following experiments. The chemical changes which took place under the standard condition were followed periodically on aliquots of the solutions. The factors investigated thus far were: The decrease of amino-nitrogen; the decrease of sugars; the increase of acidity; the production of ammonia, urea, cyanic acid, aldehydes, carbon dioxide, etc. The amino-nitrogen was determined by Van Slyke's method after the removal of ammonia by permutit, when necessary, and the sugars by the Somogyi modification of Shaffer-Hartmann's method. The presence of amino acids in amounts which were found in the present experiments, did not interfere with the sugar determination to any noticeable extent. The increase of acidity was deduced from the quantity of alkali neutralized in the reacting mixture and was expressed in terms of cc. of *N* acid. Ammonia was measured by the Van Slyke-Cullen method or by permutit-Nesslerization method, and

urea by urease method or by xanthydrol method. Cyanic acid was determined by Ewan's method (1904) or as dixanthyl urea after converting it into urea by evaporating its solution in the presence of ammonium chloride. The amount of carbon dioxide was calculated roughly from the difference in the volume of gas, which was evolved from the solution in Van Slyke's apparatus, before and after absorbing it with alkali. The possible influence of volatile acids formed on the gasometry was neglected. The production of aldehydes was estimated iodometrically in the distillate of the neutralized solution under the assumption that the substance which formed iodoform was aldehyde. The fact that the disintegration of the sugar in strong alkaline solution takes place in quite a complicated way with the production of aldehydic substances, made it impossible to determine the chemical nature of the aldehyde produced. If produced, the aldehyde might be the one with an atom less of carbon than the corresponding amino acid. Since the identification of its nature failed, the quantity of the aldehyde which increased in the presence of the amino acid was provisionally expressed in terms of cc. of $N/10$ iodine solution consumed for the formation of iodoform. The results thus obtained were only of an approximate nature, since many other substances, in addition to the aldehyde under examination, may react with iodine.

The accelerating effects of various amounts of glucose on the deamination of glycine were first studied. The experiments were carried out under the standard condition mentioned above with the variation of the quantity of glucose. The data of Table I show that the accelerating effects manifested themselves more markedly with the increasing quantities of glucose.

The results of the study on the accelerating effect of glucose on the deamination of various amino acids are shown in Table II. In these experiments the molar ratio between amino acids and glucose was 1.0:1.5 under the standard condition. It was found that the effect of glucose was shown most remarkably in the case of glutamic acid, next to which came, in the order named, glycine, alanine, leucine and lysine. The significance, if any, attaching to

TABLE I. Accelerating Effect of Glucose on Deamination of Glycine.
16.67 cc. of *M* glycine solution and various amounts of glucose
in 100 cc., 0.5*N* NaOH, 37.5°C.

Time (hrs.)	Decrease of glycine (in 100 cc.)															
	Glycine alone	Molar ratio of glycine to glucose														
		1:1			1:1.5			1:2			1:3			1:4		
		Glycine + glucose	Acceleration		Glycine + glucose	Acceleration		Glycine + glucose	Acceleration		Glycine + glucose	Acceleration		Glycine + glucose	Acceleration	
	mg.	mg.	mg.	%	mg.	mg.	%	mg.	mg.	%	mg.	mg.	%	mg.	mg.	%
2	13.82	21.16	7.34	53.1	25.29	11.47	83.0	27.27	13.75	97.3	37.45	23.63	171.0	55.13	41.31	298.9
4	28.72	41.58	12.86	44.8	45.70	16.98	59.1	54.33	25.61	89.2	69.06	40.34	140.5	90.97	62.25	216.7
6	44.79	60.11	15.32	34.2	65.17	21.38	47.7	75.60	30.81	68.8	89.96	45.17	100.8	109.14	64.35	143.7

TABLE II. Accelerating Effect of Glucose on Deamination of Various Amino Acids.
16.67 cc. of *M* amino acid and 25.01 cc. of *M* glucose solution
in 100 cc., 0.5*N* NaOH, 37.5°C.

Molar ratio of amino acids to glucose = 1.0 : 1.5.

	Decrease of amino-nitrogen (%)												Acceleration (%)					
	Amino acids alone						Amino acids + glucose											
	Time (hrs.)																	
	2	3	4	6	24	48	2	3	4	6	24	48	2	3	4	6	24	48
Glycine	1.11	—	2.30	3.58	9.76	13.71	2.02	—	3.65	5.29	13.92	19.43	82.0	—	58.7	48.0	42.6	41.9
Alanine	1.13	—	2.34	3.57	9.61	13.71	1.97	—	3.63	5.17	13.59	19.01	74.4	—	55.1	44.8	41.4	38.7
Leucine	1.13	—	2.16	3.16	8.52	13.21	1.87	—	3.35	4.72	11.73	17.90	65.5	—	55.2	49.4	37.7	35.5
Glutamic acid	—	1.73	—	3.43	9.04	12.99	—	3.39	—	5.85	14.54	19.70	—	95.7	—	70.6	60.9	51.7
Lysine	0.94	—	1.87	2.63	7.75	—	1.35	—	2.44	3.29	9.44	—	44.4	—	30.3	24.9	21.7	—

the different reactivity of amino acids is not apparent, but it may be recalled that Grünhut and Weber noticed the same order of the reactivity of amino acids towards glucose at neutral reaction and boiling temperature. The fact that among amino acids the deamination of lysine was least accelerated by glucose might be explained by the presence of two amino groups in its molecule. This circumstance may bring the same result as in the case where the quantity of the sugar is reduced by half.

The sugars of different kinds affected the deamination of amino acids to different degrees. The sugar with more sensitivity towards the action of alkali was more effective. Thus the ratio of the susceptibility of glucose, fructose, galactose and glyceraldehyde towards alkali was 1.0:2.5:0.7:2.7 respectively. If the alkali-sensitivity would directly mean the reactivity towards the amino acid, the molar ratio of the sugars of the same accelerating effectiveness should be 1.0:0.4:1.5:0.37. In the experiments shown in Table III the quantity of glucose, which corresponded to 16.67 cc. of *M* glycine solution in the molar ratio of 1.5:1.0, was taken as a unit. Then the molar ratio of the equivalent amounts of fructose, galactose and glyceraldehyde to that of glucose should be 0.6, 2.25 and 0.55 respectively. The table reveals that when the equivalent amounts of sugars were used under standard condition, their accelerating effects on various amino acids were approximately the same. This was also the case where the quantities of sugars were doubled; glucose 3.0, fructose 1.2 and galactose 4.5.

If the reaction mentioned above be conceived of as involving an actual combination between the amino and aldehyde group, it would be of interest to follow the fate of the amino group. There are perhaps two possibilities concerning the mechanism of the disappearance of the amino group: the one is the conversion into free ammonia, the other the change to imino or other groups. The existing literature shows that free ammonia is never detected in the reacting solution when the amino acid and sugar were heated at neutral reaction. Under the conditions of the present experiments the slow decrease of the amino-nitrogen of the amino acid in the absence of the sugar was not accompanied by the

TABLE III. Accelerating Effect of Various Sugars on Deamination of Amino Acids.
16.67 cc. of *M* amino acid solution in 100 cc., 0.5*N* NaOH, 37.5°C.

Sugar	Molar ratio of amino acid to sugar	Time (hrs.)	Decrease of amino-nitrogen (%)*			Acceleration (%)		
			Glycine	Alanine	Leucine	Glycine	Alanine	Leucine
Glucose	1:1.5	2	2.02	1.97	1.87	82.0	74.4	65.5
		4	3.65	3.63	3.35	58.7	55.1	52.2
		6	5.29	5.17	4.72	48.0	44.8	49.4
	1:3.0	2	2.99	—	—	171.0	—	—
		4	5.52	—	—	140.5	—	—
		6	7.19	—	—	100.8	—	—
Fructose	1:0.6	2	1.97	1.99	1.99	78.7	76.1	76.1
		4	3.79	3.55	3.34	65.1	51.7	54.6
		6	5.49	5.19	4.74	53.3	45.4	50.1
	1:1.2	2	3.01	—	—	172.5	—	—
		4	5.21	—	—	126.7	—	—
		6	7.30	—	—	103.7	—	—
Galactose	1:2.25	2	—	1.99	1.81	—	76.1	60.2
		4	—	3.64	3.36	—	55.7	55.2
		6	—	5.19	4.75	—	45.2	50.5
	1:4.5	2	2.90	—	—	162.5	—	—
		4	5.38	—	—	134.1	—	—
		6	7.26	—	—	102.8	—	—
Glyceraldehyde	1:0.56	2	—	2.02	1.94	—	79.8	71.9
		4	—	3.69	3.33	—	57.9	54.1
		6	—	5.21	4.72	—	45.8	49.5

* The decrease of amino-nitrogen in the absence of the sugar has been shown in Table II.

production of ammonia, urea, cyanic acid, aldehyde or carbon dioxide. The addition of the sugar caused, however, besides the acceleration of the deamination, the formation of ammonia, aldehyde and carbon dioxide. No production of urea or cyanate was found. The production of ammonia was observed repeatedly in a convincing way, though its quantity did never exceed 15% of the amino-nitrogen, the disappearance of which was caused by the addition of the sugar. Glycine was an exception. The amount of ammonia formed from it accounted for more than 60% of the amino-nitrogen decreased by the presence of the sugar. The liberation of free ammonia seems to suggest the occurrence of the deamination, in the ordinary sense at least, is a part of the reaction. The greater part of the free ammonia, if produced, might combine with glucose or with its decomposition-products and might form melanoidins. The amounts of aldehydes and carbon dioxide which were also produced from sugars in the absence of amino acids, increased obviously when amino acids were added to them. Glycine was again an exception in the production of carbon dioxide and aldehyde. The amount of carbon dioxide increased by the addition of the amino acids other than glycine covered at most 15% of the amino acid which disappeared, while glycine produced a much larger quantity of carbon dioxide. Moreover, no increase of aldehyde was observed in this case. The same phenomenon was noticed by Yamada, and Ambler in their experiments of heating the solution of glycine and glucose. Ambler explained this fact by assuming that formaldehyde which should have actually been produced from glycine, might condense rapidly and completely with amino compounds. In the present experiments glutamic acid resembled glycine in that it did not give rise to the production of aldehyde. Neuberg and Kobel (1927 iii) demonstrated that methylglyoxal reacted with amino acids and formed aldehydes which corresponded to amino acids. They failed, however, to show the production of formaldehyde from glycocoll. The abnormal behaviour of glycine was also observed in the experiments of oxidizing amino acids with alloxan. Strecker (1862) found that on warming with the solution of alloxan, the solution of leucine or

alanine produced isovaleraldehyde or acetaldehyde respectively with the simultaneous formation of CO_2, while glycine gave no aldehyde but did give CO_2. Hurtley and Wootton (1911) confirmed this finding in the concentrated solution. They proved, however, the presence of formaldehyde in the distillate obtained from a dilute solution. Hill (1932) referred to detected the presence of formaldehyde in the solution of glycine which had been oxidized by alloxan.

Contrary to the fact that the sugar accelerated the deamination in alkaline solution, the disintegration of the sugar by alkali was retarded by the presence of the amino acid to a noticeable extent. The production of lactic acid and other acids was also inhibited as a natural consequence of the retardation of the sugar decomposition. The explanation of this inhibitory effect of amino acid on the sugar decomposition requires the elucidation of the chemical nature of the reaction. Löb and his co-workers (1912; 1915) observed the retarding effect of glycine on the oxidation of glucose by hydrogen peroxide in the presence of phosphate.

Illustrative figures for all the evidence concerning the production of ammonia, aldehydes, carbon dioxide, lactic acid and other acids are given in Tables IV–XIV. The experiments followed in principle the same routine as in those listed in the foregoing tables. To 16.67 cc. of *M* solutions of various amino acids were added certain quantities of various sugars. The amino acids used were glycine, alanine, leucine and glutamic acid; the sugars glucose, fructose, galactose and glyceraldehyde. The molar ratio of amino acids to the quantities of glucose, fructose, galactose and glyceraldehyde were 1.0 : 1.5, 1.0 : 0.6, 1.0 : 2.2 and 1.0 : 0.55 respectively, the amounts of the sugars having been chosen so as to have the same accelerating effects on the deamination of amino acids.

The Reaction at pH 11.9, 10.4 and 9.2.

By lowering the alkalinity of the medium it was intended to see whether the reaction might also take place under the conditions where the spontaneous decomposition of the reacting substances was reduced to the minimum extent. For this purpose a series of phos-

TABLE IV. Action of Glucose on Glycine.

16.67 cc. of *M* glycine solution in 100 cc., 0.5*N* NaOH, 37.5°C.
Molar ratio of glycine to glucose = 1.0 : 1.5.

Time (hrs.)	Decrease of glycine				Decrease of glucose		Increase of acidity		Increase of lactic acid		Production of NH_3		Production of CO_2		Production of aldehyde	
	Glycine alone	Glycine + glucose	Acceleration		Glucose alone	Glucose + glycine	Glucose alone	Glucose + glycine	Glucose alone	Glucose + glycine	Glycine alone	Glycine + glucose	Glucose alone	Glucose + glycine	Glucose alone	Glucose + glycine
	mg.		mg.	%	mg.		cc. *N* acid		mg.		mg.		mg.		cc. 0.1 *N* iodine	
2	13.82	25.29	11.47	83.0	393.0	286.0	3.24	2.03	95.79	51.02						
4	28.72	45.70	16.98	59.1	780.0	634.0	7.71	6.09	178.21	120.74						
6	44.79	66.17	21.38	47.7	1220.0	1088.0	11.36	9.33	288.11	199.18	0	3.25				
24	121.99	174.07	52.08	42.8	3260.0	2968.0	30.45	24.56	613.77	443.54	0	7.67	12.00	28.62	32.20	30.20
48	171.50	242.97	71.47	41.5	3978.0	3371.0	34.78	32.91			0	10.21	17.75	35.83	34.21	31.40

TABLE V. Action of Glucose on Alanine.

Time (hrs.)	Decrease of alanine				Decrease of glucose		Increase of acidity		Production of NH_3	Production of CO_2		Production of aldehyde	
	Alanine alone	Alanine + glucose	Acceleration		Glucose alone	Glucose + alanine	Glucose alone	Glucose + alanine	Alanine + glucose	Glucose alone	Glucose + alanine	Glucose alone	Glucose + alanine
	mg.		mg.	%	mg.		cc. N acid		mg.	mg.		cc. 0.1N iodine	
2	16.72	29.25	12.53	74.9	393.0	128.0	3.24	1.96					
4	34.71	53.85	19.14	55.1	780.0	532.0	7.71	4.51					
6	53.02	76.68	23.66	44.6	1220.0	820.0	11.36	7.85	0.58				
24	142.54	201.61	59.05	41.4	3260.0	2360.0	30.45	26.72	1.01	12.05	14.72	32.20	44.60
48	203.39	282.16	78.77	38.7	3978.8	3403.0	34.70	31.42	1.48	12.75	17.33	34.21	52.38

TABLE VI. Action of Glucose on Leucine.

Time (hrs.)	Decrease of leucine				Decrease of glucose		Increase of acidity		Production of NH_3	Production of CO_2		Production of aldehyde	
	Leucine alone	Leucine + glucose	Acceleration		Glucose alone	Glucose + leucine	Glucose alone	Glucose + leucine	Leucine + glucose	Glucose alone	Glucose + leucine	Glucose alone	Glucose + leucine
	mg.		mg.	%	mg.		cc. N acid		mg.	mg.		cc. 0.1N iodine	
2	24.62	40.81	16.19	65.8	393.0	210.0	3.24	1.81					
4	47.27	73.29	26.02	55.0	780.0	515.0	7.71	4.04					
6	68.98	103.14	34.16	49.5	1220.0	820.0	11.36	7.44					
24	186.16	256.17	82.18	37.7	3260.0	2238.0	30.45	26.59	0.55	12.00	17.34	32.20	42.0
48	288.56	391.14	102.58	35.5	3978.0	3308.0	34.78	32.13	0.63	17.75	23.86	34.21	44.4

TABLE VII. Action of Glucose on Glutamic acid.

Time (hrs.)	Decrease of glutamic acid				Decrease of glucose		Increase of acidity		Production of NH_3	Production of CO_2		Production of aldehyde	
	Glutamic acid alone	Glutamic acid + glucose	Acceleration		Glucose alone	Glucose + glutamic acid	Glucose alone	Glucose + glutamic acid	Glutamic acid + glucose	Glucose alone	Glucose + glutamic acid	Glucose alone	Glucose + glutamic acid
	mg.		mg.	%	mg.		cc. N acid		mg.	mg.		cc. 0.1N iodine	
3	43.78	85.68	41.89	95.7	631.0	454.0	5.61	3.92					
6	83.73	148.05	61.32	70.7	1220.0	973.7	11.36	7.85					
24	228.68	368.01	139.33	60.9	3260.0	2702.4	30.54	25.54	0.65	12.00	19.07	32.20	29.00
48	328.64	498.42	169.78	51.7	3978.0	3371.0	34.78	31.04	1.06	12.75	24.44	34.21	31.00

TABLE VIII. Action of Fructose on Glycine.

16.67 cc. of M glycine solution in 100 cc., 0.5N NaOH, 37.5°C. Molar ration of glycine to fructose = 1.0 : 0.6

Time (hrs.)	Decrease of Glycine				Decrease of fructose		Increase of acidity		Production of NH_3	Production of CO_2		Production of aldehyde	
	Glycine alone	Glycine + fructose	Acceleration		Fructose alone	Fructose + glycine	Fructose alone	Fructose + glycine	Glycine + fructose	Fructose alone	Fructose + glycine	Fructose alone	Fructose + glycine
	mg.		mg.	%	mg.		cc. N acid		mg.	mg.		cc. 0.1N iodine	
2	13.82	24.59	10.77	78.7	425.6	350.5	3.85	3.66					
4	28.72	47.42	18.70	65.1	654.1	563.2	6.28	5.28					
6	44.79	68.69	23.90	53.3	847.0	748.3	8.92	8.12					
24	121.99	151.09	29.09	23.8	1545.1	1424.8	12.58	12.38	4.46	5.03	9.18	17.40	14.00
48	171.50	204.45	32.95	19.2	1678.2	1633.8	16.64	16.65	5.74	6.47	16.53	12.02	10.00

TABLE IX. Action of Fructose on Alanine.

Time (hrs.)	Decrease of alanine				Decrease of fructose		Increase of acidity		Production of NH_3	Production of CO_2		Production of aldehyde	
	Alanine alone	Alanine + fructose	Acceleration		Fructose alone	Fructose + alanine	Fructose alone	Fructose + alanine	Alanine + fructose	Fructose alone	Fructose + alanine	Fructose alone	Fructose + alanine
	mg.		mg.	%	mg.		cc. N acid		mg.	mg.		cc. 0.1N iodine	
2	16.72	29.56	12.84	76.8	425.6	324.9	3.95	3.65					
4	34.71	52.64	17.93	51.6	654.1	512.5	6.28	5.27					
6	53.02	76.99	23.97	45.2	847.0	687.6	8.92	7.71	0.34				
24	142.54	167.72	25.16	17.7	1515.1	1373.0	12.58	12.37	0.54	5.03	6.46	17.40	23.60
48	203.39	233.46	30.07	14.8	1678.2	1636.9	16.64	16,64	0.75	6.47	8.21	12.02	20.01

TABLE X. Action of Fructose on Leucine.

Time (hrs.)	Decrease of leucine				Decrease of fructose		Increase of acidity		Production of NH_3	Production of CO_2		Production of aldehyde	
	Leucine alone	Leucine + fructose	Acceleration		Fructose alone	Fructose + leucine	Fructose alone	Fructose + leucine	Leucine + fructose	Fructose alone	Fructose + leucine	Fructose alone	Fructose + leucine
	mg.		mg.	%	mg.		cc. N acid		mg.	mg.		cc. 0.1N iodine	
2	24.62	43.52	18.91	76.8	425.6	320.7	3.85	3.65					
4	47.27	72.91	25.64	54.3	654.1	551.4	6.28	5.07					
6	68.98	103.52	34.54	50.1	847.0	717.0	8.92	7.51					
24	186.16	220.79	34.63	18.6	1545.1	1425.6	12.58	12.38	0.29	5.03	6.49	17.40	24.00
48	288.56	326.93	38.37	13.3	1678.2	1608.0	16.64	16.44	0.41	6.47	10.58	12.02	25.01

TABLE XI. Action of Galactose on Alanine.

16.67 cc. of *M* alanine solution in 100 cc., 0.5*N* NaOH, 37.5°C. Molar ratio of alanine to galactose = 1.0 : 2.2.

Time (hrs.)	Decrease of alanine				Decrease of galactose		Increase of acidity		Production of NH_3	Production of CO_2		Production of aldehyde	
	Alanine alone	Alanine + galactose	Acceleration		Galactose alone	Galactose + alanine	Galactose alone	Galactose + alanine	Alanine + galactose	Galactose alone	Galactose + alanine	Galactose alone	Galactose + alanine
	mg.		mg.	%	mg.		cc. *N* acid		mg.	mg.		cc. 0.1*N* iodine	
2	16.72	29.50	12.78	76.4	370.3	311.6	2.53	2.43					
4	34.71	54.04	19.33	55.7	825.5	695.2	4.96	4.85					
6	53.02	76.99	23.97	45.2	1122.9	973.8	7.59	7.17					
24	142.54	203.51	60.97	42.8	3732.7	2498.9	27.81	21.18	0.77	3.69	7.46	36.00	50.00
48	203.39	302.38	98.99	48.7	5153.1	3835.3	37.51	30.66	0.97	7.55	12.96	34.00	48.00

TABLE XII. Action of Galactose on Leucine.

Time (hrs.)	Decrease of leucine				Decrease of galactose		Increase of acidity		Production of NH_3	Production of CO_2		Production of aldehyde	
	Leucine alone	Leucine + galactose	Acceleration		Galactose alone	Galactose + leucine	Galactose alone	Galactose + leucine	Leucine + galactose	Galactose alone	Galactose + leucine	Galactose alone	Galactose + leucine
	mg.		mg.	%	mg.		cc. *N* acid		mg.	mg.		cc. 0.1*N* iodine	
2	24.62	39.59	14.98	60.9	370.3	310.8	2.53	3.05					
4	47.27	73.38	26.11	55.2	825.5	665.8	4.96	4.74					
6	68.98	103.80	34.82	50.5	1122.9	914.4	7.59	6.95					
24	186.16	286.22	100.15	53.7	3732.7	2958.4	27.81	21.49	0.42	3.69	11.13	36.00	48.99
48	288.56	413.41	124.86	43.3	5153.1	4129.7	37.51	32.55	0.72	7.55	14.71	34.00	54.00

TABLE XIII. Action of Glyceraldehyde on Alanine.

16.67 cc. of *M* alanine solution in 100 cc., 0.5*N* NaOH, 37.5°. Molar ratio of alanine to glyceraldehyde = 1.0 : 0.55.

Time (hrs.)	Decrease of alanine				Decrease of glyceraldehyde		Increase of CO_2		Production of NH_3	Production of CO_2		Production of aldehyde	
	Alanine alone	Alanine + glyceraldehyde	Acceleration		Glyceraldehyde alone	Glyceraldehyde + alanine	Glyceraldehyde alone	Glyceraldehyde + alanine	Alanine + glyceraldehyde	Glyceraldehyde alone	Glyceraldehyde + alanine	Glyceraldehyde alone	Glyceraldehyde + alanine
	mg.		mg.	%	mg.		cc. *N* acid		mg.	mg.		cc. 0.1*N* iodine	
2	16.72	30.07	13.35	79.8	220.8	184.9	3.25	3.04					
4	34.71	54.80	20.09	57.9	378.6	331.7	4.67	4.06					
6	53.02	77.31	24.29	45.8	518 7	443.2	5.68	5.27					
24	142.54	162.89	20.34	14.3	788.7	748.8	8 53	7.71	0.56	5.31	8.99	7.00	13.51

TABLE XIV. Action of Glyceraldehyde on Leucine.

Time (hrs.)	Decrease of leucine				Decrease of glyceraldehyde		Increase of acidity		Production of NH_3	Production of CO_2		Production of aldehyde	
	Leucine alone	Leucine + glyceraldehyde	Acceleration		Glyceraldehyde alone	Glyceraldehyde + leucine	Glyceraldehyde alone	Glyceraldehyde + leucine	Leucine + glyceraldehyde	Glyceraldehyde alone	Glyceraldehyde + leucine	Glyceraldehyde alone	Glyceraldehyde + leucine
	mg.		mg.	%	mg.		cc. *N* acid		mg.	mg.		cc. 0.1*N* iodine	
2	24.62	42.31	17.69	71.9	220.8	192.7	3.25	3.25					
4	47.27	72.82	25.55	54.1	378.6	327.1	4.67	4.47					
6	68.98	103.14	34.16	49.5	518.7	441.1	5.68	5.28					
24	186.16	215.18	29.01	15.6	788.7	745.7	8.53	7.71	0.37	5.31	7.96	7.00	12.50

phate-NaOH buffer solutions of high capacity was prepared. The quantity of the amino acid and glucose was reduced to 1/5 of the case at 0.5*N* alkalinity, to avoid the lowering of pH of the buffer solutions by the acids produced from the sugar. The mixture of 3.33 cc. of *M* alanine solution and 5.0 cc. of *M* glucose solution (molar ratio = 1.0 : 1.5) was diluted to 100 cc. with the buffer solutions mentioned above, the pH-values of which were 11.9, 10.4 and 9.2 respectively. The interaction of alanine and glucose was examined periodically at 50°. These alkalinities were still high enough to cause the spontaneous dissolution of the reacting substances to a notable degree. It was observed that the accelerating effect of glucose on the deamination manifested itself more markedly at lower alkalinity. This phenomenon might be partly explained by the fact that at lower alkalinity the rate of the decomposition of glucose became relatively slower as compared with that of alanine; in other words, the circumstances resembled the case where a relatively large amount of glucose was used, thus accelerating the deamination more strongly. It is to be noted that the production of ammonia was also observed definitely at these alkalinities. The decrease of glucose was retarded by the presence of alanine. The results are summarized in Table XV.

A few remarks are inserted here on the experiments carried out by Euler and his collaborators. According to Euler, Brunius and Josephson's statement (1926) the reaction between the amino acid and sugar was reversible even at pH 10.4 alkalinity. They found that the solution which contained 15 cc. of *M* glucose, 15 cc. of 2 *M* alanine and 11 cc. of 2*N* NaOH solution in 50 cc. restored almost completely its glucose which had decreased during 41 hours' incubation at room temperature, when the solution was acidified and left aside for 25 hours. The present writer found, however, that this was not the case. No regeneration of the decreased glucose or alanine was noticed at all under strictly the same experimental conditions as were described by Euler and his coworkers. Thus the reaction at pH 10.4 and 20°C were irreversible. The Swedish investigaters made another observation that the preliminary treatment of glucose with alkali did not result in the

TABLE XV. Action of Glucose on Alanine at Various pH.

3.33 cc. of *M* alanine and 0.5 cc. of *M* glucose solution in 100 cc., phosphate-NaOH buffer, 50°C.

Molar ratio of alanine to glucose = 1.0 : 1.5.

pH	Time (day)	Decrease of alanine				Decrease of glucose		Production of ammonia
		Alanine alone	Alanine + glucose	Acceleration		Glucose alone	Glucose + alanine	
		mg.		mg.	%	mg.		mg.
11.9	1/6	4.20	17.87	13.67	325.5	422.7	345.0	—
	1/3	8.01	31.15	23.14	288.9	828.5	510.2	0.40
	1	18.95	36.24	17.29	91.2	880.2	700.5	0.61
	2	33.51	46.86	13.35	39.8	880.2	831.3	—
10.4	1	7.38	49.65	42.28	572.9	570.6	379.3	—
	2	14.75	80.49	65.74	445.7	616.0	469.2	0.75
	3	19.39	97.97	78.85	405.3	643.2	567.7	0·97
	5	29.82	110.43	80.62	270.4	668.8	668.2	1.03
	7	37.57	118.06	80.49	214.2	700.0	685.0	1.46
9.2	1	2.23	29.37	27.14	1217.5	65.6	40.8	—
	2	4.20	41.96	37.76	899.3	111.2	94.4	1.03
	3	6.10	49.59	43.49	716.1	135.6	132.4	1.36
	5	10.11	62.56	52.45	518.8	183.2	176.2	1.75
	7	14.37	74.89	60.52	421.2	231.2	230.4	—

increase of its reactivity towards alanine when examined cryoscopically. Upon testing this observation it was found that by the alkali-treatment glucose gained a slight but definite increase of reactivity. This increase of reactivity was noticed chemically first after the treated sugar had been brought to contact with alanine for some time. A mixture of 15 cc. of *M* glucose and 5 cc. of 2*N* NaOH solution lost 208 mg. of glucose (1.9%) and 0.25 cc. of *N* NaOH after 18 hours' incubation at 20°C (instead of 17°C in Euler's experiment). Solution A was made by adding 15 cc. of *M* alanine solution to 20 cc. of the alkali-treated sugar solution. Solution B was prepared by adding 15 cc. of *M* alanine to the

solution which contained the same amount of NaOH and the non-treated glucose as A. The decrease of alanine in Solution A was 4.4 and 10.5 mg. after the incubation at 20° for 8 and 30 hours, while the content of alanine in Solution B remained practically constant during the incubation. When more profound decomposition of glucose was caused by the previous treatment, the reactivity of glucose increased to a higher extent. One gm. of glucose, dissolved in 100 cc. of pH 9.7 buffer solution, was boiled for 30 minutes. By this treatment, 86% loss of the glucose resulted with a concomitant appearance of the reducing power in the cold. 50 cc. of this solution were mixed with 16.67 cc. of *M* glycine solution, diluted to 100 cc. with water and kept at 20°C. A definite decrease of glycine and the production of ammonia were noticed in a relatively short time while the non-treated glucose never reacted with glycine under the same condition (Table XVI).

Table XVI. Action of Alkali-treated Glucose on Glycine.

16.67 cc. of *M* glycine solution and 50 cc. of 1% glucose solution which was boiled for 30 minutes at pH 9.7, were diluted to 100 cc., 20°C.

Time (hrs.)	Decrease of glycine (mg.)		Production of ammonia (mg.)	
	With untreated glucose	With alkali-treated glucose	With untreated glucose	With alkali-treated glucose
0.5	0	3.43	—	—
1	0	6.86	—	—
2	0	11.79	—	—
4	0	15.70	0	2.75
7	0	21.70	0	3.96

The fact that previous treatment of the sugar with alkali increases its reactivity towards the amino acid, can be understood under the assumption that the "activated sugar" or the partial decomposition-products of the sugar, such as active trioses or methylglyoxal, might be, at least in part, responsible for the irreversible reaction. There is another possible mechanism of the reaction in the alkaline medium; that is, the direct interaction between the amino acid and sugar. The reaction of this type has

been repeatedly observed by numerous investigators in the experiments at neutral reaction and high temperature. It has never been, however, demonstrated definitely that the sugar can really react in its unchanged form with the amino acid. To settle this problem experimental condition were sought under which the sugar was still unchanged, yet was capable of reacting with the amino acid. For this purpose the watery solution containing the amino acid and sugar were heated at 100°C.

Reaction in Watery Solution.

To 16.67 cc. of *M* glycine or alanine solution were added 25.0 cc. of *M* glucose or fructose solution (molar ratio=1.0:1.5). The resultant mixtures were made up to 100 cc. with water and boiled under the reflex condenser. In blank experiments, where amino acids or sugars alone were treated in the same way as in the main experiments, it was found that glucose first showed indication of decomposition after 48 hours' heating while fructose had far less resistance against heating. The amino acids remained unchanged even after 120 hours. The pH value of the glucose solution was 6.0 at the beginning and fell down to 4.8 at the end of 120 hours' boiling. The solutions of amino acids kept the same pH throughout the heating period. It should be emphasized that in the mixtures of amino acids and glucose a 7 hours' heating caused already a distinct decrease of both substances, which became more obvious with the prolongation of the time of heating. pH of the glucose-glycine solution fell from pH 6.2 to 4.2 during 120 hours, while the fall was from pH 6.5 to 4.3 in the glucose-alanine solution. In the case of alanine plus fructose, both substances showed 1.7 times more decrease than the corresponding case of alanine and glucose. The reactions under these conditions were found also to be irreversible. No increase of free sugar or amino acid was observed by leaving the reaction mixtures alone, which had been either acidified or not. The results are shown in Table XVII. The molecular ratio between the decreased quantities of amino acids and sugars was always strictly 1:1 under the conditions of the experiments, showing that one molecule of glucose or fructose

TABLE XVII. Interaction of Amino Acids and Sugars in Watery Solution.

16.67 cc. of *M* amino acid solution and 25.0 cc. of *M* sugar solution.
Molar ratio of amino acid to sugar = 1.0 : 1.5.

Time of heating (hrs.)	Decrease of glycine or alanine alone (mg.)	Decrease of glucose alone (mg.)	Decrease of fructose alone (mg.)	Glycine + glucose			Alanine + glucose			Alanine + fructose		
				Decrease of glycine (mg.)	Decrease of glucose (mg.)	Mol. ratio	Decrease of alanine (mg.)	Decrease of glucose (mg.)	Mol. ratio	Decrease of alanine (mg.)	Decrease of fructose (mg.)	Mol. ratio
7	0	0	21.0	23.09	55.0	1:0.99	23.33	50.0	1:1.03	43.68	105.9	1:0.95
22	0	0	73.4	71.53	180.0	1:1.05	74.13	150.0	1:1.00	118.26	314.5	1:1.01
48	0	15.0	167.7	164.16	405.0	1:0.99	156.15	325.0	1:0.98	234.28	649.9	1:1.02
72	0	26.0	—	228.72	550.0	1:0.96	221.19	472.5	1:1.00	—	—	—
120	0	45.0	—	379.22	985.0	1:1.03	376.32	792.0	1:0.98	—	—	—

reacted with one molecule of glycine or alanine. Diverse findings in the quantitative ratio between the reacting amino acid and sugar are to be found in the literature. From the results of their studies on the reversible reaction of amino acids with glucose at neutral reaction and low temperature, Euler and Brunius (1921 ii) concluded that one molecule of the monoamino acid should react with one molecule of the sugar Ruckdeschel assumed, however, that two molecules of glucose might combine with a molecule of the amino acid. Maillard stated that several mols of glucose must have been involved. Ambler considered that the loss of reducing sugars caused by amino acids might amount possibly to 10 or more mols per mol of amino acids.

It was thus shown that the amino acid was capable of reacting directly with the unchanged sugar. However, the question whether the same reaction which is noticed in the neutral medium and at high temperature, might also take place in the alkaline medium and at lower temperature is as yet uncertain.

Conclusion.

All facts considered, the reaction between the amino acid and sugar is of irreversible nature, so far as the reaction is deep-seated enough to be followed in a chemical way. When the sugar which is in process of disintegration is employed, the reaction seems to take place in a complicated way and its mechanism is not clear. There might be two possible modes of the reaction. The one is the direct interaction between the unchanged sugar and the amino acid, the other is the participation of the "activated sugar" or of the partial decomposition-products of the sugar into the reaction. Whenever the sugar was added to the solution of the amino acid at any alkalinity, the mixture became, as has been often observed at neutral reaction and high temperature, brown coloured much more rapidly than in the case of comparable solutions of any of the components. This must be due to the production of "melanoidins". The obscurity in which the chemistry of the interaction of the amino acid with sugar is shrouded may be much clarified when there is sufficient knowledge about the chemical

nature of melanoidins and the fate of the sugar involved in the reaction.

Summary.

1. The deamination of amino acids in alkaline solution (0.5*N* sodium hydroxide solution; pH 11.9, 10.4 and 9.2) was accelerated remarkably by the presence of sugars. The acceleration was accompanied by the production of ammonia, carbon dioxide and aldehydes from amino acids. The accelerating action of various kinds of sugars was examined on various amino acids. The sugar which has weaker resistance against alkali was more effective. The previous treatment of the sugar with alkali resulted in a slight increase of reactivity towards the amino acid. The presence of the amino acid retarded the disintegration of the sugar in the alkaline solution. The accelerating effect of the sugar on the deamination of the amino acid manifested itself more markedly at lower alkalinity.

2. In a watery solution and at boiling temperature, the sugar and amino acid reacted with one another at the equimolecular ratio at an early stage of the reaction, where the sugar or the amino acid alone did not show even an indication of decomposition.

3. The reactions both in alkaline and neutral medium were irreversible, so far as they were deep-seated enough to be followed by chemical methods.

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STUDIES IN EXPERIMENTAL SCURVY.

XIV. On the Content of Fatty Acid and Lipins in Blood of Guinea Pigs Fed on a Vitamin C Free Diet.

By

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Introduction.

According to Collazo and Basch (1924), in the course of avitaminosis C, the content of blood fat increases although at the end there is shown a slight diminution. In this case, cholesterol is also parallel to the content of fat, while in phospholipin no change is seen at the beginning but there is seen a slight decrease at the end of scurvy. Ssakolow (1925) reported that cholesterol content in the blood of scorbutic guinea pigs decreases gradually and the rate of the decrease runs parallel to the severity of the disease and as soon as they come to recovery it reaches the normal value. On the other hand, Moriquand, Leulier, Michel and Idroe advocate that in both acute and chronic avitaminosis C, cholesterol amount is not altered at all. Fukuda (1929) proved that blood fat begins to increase with the loss of body weight, and the alcohol extract is mainly increased.

As stated above, not only have there been shown various results in the blood lipins of scorbutic animals, but also these investigations are fragmentary.

From these points of view, we made a series of experiments and determined higher fatty acid, phospholipin, and cholesterol in blood in both normal and scorbutic cases.

Experimental Animals.

After the preliminary experiment it has been found necessary

to use animals healthy and weighty enough to stand blood drawing for six times by 15–16 drops each time. Therefore, in the present experiment, guinea pigs weighing over 150 gm were employed. All of them were fed for some ten days with a definite diet, and for a few days before the experiment they were fed in metabolic cages with oat-meal and fresh radish roots.

Feeding.

Previously we estimated blood cholesterol content in experimental scurvy which has taken place in guinea pigs reared with Sherman's diet. In that case, as we were not able to secure a reliable result on account of intolerable fluctuation of the cholesterol content even in normal animals, we planned to ascertain what the fluctuation is due to.

We had a chance to get Sato's report (1927) on the fate of cholesterol in the serum of guinea pigs fed on whole milk or Morinaga's powdered milk. According to his report, it is proved that those animals fed on powdered milk show greater fluctuability than the others. For this reason, in the present experiment we are obliged to use simply oatmeal instead of Sherman's diet. It is very difficult to rear the animals with simply oatmeal and radish juice. When the animals got used to these rations, the experiment could be carried out. As the vitamin C supply, fresh radish juice was used. For scorbutic period, it was replaced by the juice boiled at 100°C for 1½ hours.

Blood Drawing.

Blood was drawn three times in both normal and scorbutic periods from each guinea pig. In the former case, the blood was drawn every other 3 days, while in the latter it was taken every other 5 days. Some of the scorbutic animals died often after the first bleeding and some of them enabled us to draw blood.

After the ear-lobe was wiped well with alcohol and the vein was swelled by toluene, blood was drawn by piercing the peripheral side of the auriculary vein while the central side of the vein was prepared for another time when the blood drawing would become

difficult. As soon as the blood was absorbed by a good piece of absorbent paper, about 16 × 18 mm in size, it was weighed by torsion balance. The amount of blood adequate for the determination is from 85 to 100 mg.

Experimental Methods.

In the present experiment we employed Takahata-Nakamura's microdetermination method (1926) for fatty acid and lipins in blood, which needs only a minute amount of blood and will give satisfactory results.

Experimental Results.

These data are shown in Tables from I to XI. At the second bleeding, in scorbutic period, i. e., 10 days after they had been fed on vitamin C free diet, there have already been seen some scorbutic symptoms such as looseness of teeth, swelling of joints, etc. The autopsy findings are shown in the tables.

As is clear in Table XI, the average amount of total fatty acid determined 30 times in each normal and scorbutic period with 10 guinea pigs, is 0.1637% in normal while in avitaminosis C, it is 0.1918%. At the end of the scorbutic period, however, there has been shown nearly the same value as in normal. The content of phospholipin in the blood is averaged by 0.0629% in normal period while in the scorbutic it is 0.0656%. In this case we can hardly recognise any marked difference from that in normal period excepting a slight decrease shown in the advanced stage of scurvy. These results rather coincide with those reported by Collazo. The cholesterol contents of blood in normal and scorbutic periods are 0.1004% and 0.0977% on an average respectively. There is no change of cholesterol content in the blood of the guinea pigs which showed remarkable scorbutic symptoms. At the end of scorbutic period, however, there can be seen a tolerable decrease in it. This fact does not agree with Ssoklow's report—that "cholesterol content in blood decreases parallel with the severity of the disease." But on the other hand, it seems to agree rather with Michel and Idroe's claim in which they state that there is no marked

No. 69. TABLE I.

Date	Body wt.	Amt. of diet ingested	Loss of body wt.	Total fatty acid	Phospho-lipin	Cho-lesterol	Cholesterol / Phospholipin	Cholesterol / Total fatty acid	Total fatty acid / Phospholipin	Phospholipin / Cholesterol	Remarks
April	gm	gm	%	%	%	%					
25	540	16									Looseness of teeth Hemorrhages of joints
26	520	19									
27	500	21	7.4	0.1590	0.0609	0.0980	1.6092	0.6164	2.6149	0.6215	
28	500	23									
29	490	25									
30	480	21									
May											
1	450	13	17.0	0.1624	0.0324	0.0998	1.5994	0.6145	2.6026	0.6253	Fed with scorbutic diet from 6/V.
2	455	25									
3	435	16									
4	435	22									
5	435	16	19.4	0.1668	0.0639	0.1036	1.6213	0.6211	2.6103	0.6071	
6	440	24									
7	435	25									
8	435	21									
9	435	22									
10	440	18	18.5	0.2223	0.0789	0.1191	1.5095	0.5358	2.8175	0.6625	
11	425	10									
12	410	8									
13	400	13									
14	380	13									
15	370	13									
16	380	14	28.0	0.2057	0.0698	0.1052	1.5072	0.5114	2.9469	0.6635	
17	380	20									
18	380	16									
19	375	15									
20	365	12									
21	365	11									
22	365	13									
23	355	11									
24	350	9	35.1	0.1711	0.0551	0.0809	1.4428	0.4728	3.1053	0.6811	

No. 70.

TABLE II.

Date	Body wt.	Amt. of diet ingested	Loss of body wt.	Total fatty acid	Phospholipin	Cholesterol	Cholesterol / Phospholipin	Cholesterol / Total fatty acid	Total fatty acid / Phospholipin	Phospholipin / Cholesterol	Remarks
April	gm	gm	%	%	%	%					
28	500	14		0.1482	0.0567	0.0910	1.6049	0.6140	2.6134	0.6231	Looseness of teeth. Enlargement and hemorrhages of rib junction.
29	465	5									
30	450	16									
May											
1	460	15									
2	460	12	8.0	0.1731	0.0664	0.1062	1.5994	0.6193	2.6069	0.6252	
3	450	11									
4	445	12									
5	445	10									
6	445	15	11.0	0.1829	0.0702	0.1125	1.6026	0.6206	2.6054	0.624	Fed with scorbutic diet from 12/V.
7	430	12									
8	425	10									
9	420	12									
10	400	9									
11	400	17	20.0	0.2011	0.0738	0.1113	1.5217	0.5535	2.7249	0.6631	
12	410	14									
13	405	20									
14	405	23									
15	400	18									
16	405	22	19.0	0.1878	0.0658	0.0989	1.5030	0.5266	2.8541	0.6653	
17	395	18									
18	390	14									
19	365	5									
20	350	4									
21	350	6									
22	335	14									
23	315	16									
24	305	6	39.0	0.1331	0.0618	0.0887	1.4353	0.6664	2.1537	0.6967	

No. 74. TABLE III.

Date	Body wt.	Amt. of diet ingested	Loss of body wt.	Total fatty acid	Phospho-lipin	Cho-lesterol	Cholesterol / Phospholipin	Cholesterol / Total fatty acid	Total fatty acid / Phospholipin	Phospholipin / Cholesterol	Remarks
May	gm	gm	%	%	%	%					
14	500	17									
15	485	16	3.0	0.1392	0.0539	0.0864	1.6029	0.6207	2.5826	0.6227	Looseness of teeth. Enlargement and hemorrhages of rib junction.
16	485	13									
17	485	10									
18	485	15	3.0	0.1190	0.0572	0.0896	1.5664	0.6154	2,6049	0.6384	Hemorrhages of caecum.
19	485	13									
20	485	12									
21	475	13	5.0	0.1741	0.0669	0.1075	1.6069	0.6175	2.6039	0.6223	Fed with scorbutic diet from 22/V.
22	475	11									
23	465	9									
24	460	9									
25	450	16									
26	445	15									
27	450	16									
28	440	16	12.0	0.1711	0.0702	0.1150	1.6382	0.6721	2.4376	0.6104	
29	445	18									
30	445	16									
31	435	16									
June											
1	430	15									
2	425	15	15.0	0.2065	0.0676	0.1017	1.5044	0.4925	3.0547	0.6647	
3	430	15									
4	425	13									
5	410	15									
6	420	19									
7	410	14									
8	405	14									
9	370	16	26.0	0.1873	0.0573	0.0825	1.4398	0.4404	3.2688	0.6945	

No. 75. TABLE IV.

Date	Body wt.	Amt. of diet ingested	Loss of body wt.	Total fatty acid	Phospho-lipin	Cho-lesterol	Cholesterol / Phospholipin	Cholesterol / Total fatty acid	Total fatty acid / Phospholipin	Phospholipin / Cholesterol	Remarks
May	gm	gm	%	%	%	%					
14	605	17									
15	585	10	3.3	0.1629	0.0526	0.1007	1.6086	0.6182	2.6022	0.6216	Loosenes of teeth. Enlargement and hemorrhages of rib junction.
16	560	11									
17	550	12									
18	555	25	8.2	0.1663	0.0639	0.1025	0.6041	0.6164	2.6025	0.6234	
19	540	25									
20	535	25									
21	520	28	14.0	0.1757	0.0676	0.1079	1.5962	0.6141	2.5991	0.6265	Fed with scorbutic diet from 22/V.
22	515	16									
23	520	16									
24	500	15									
25	495	23									
26	485	30									
27	490	25									
28	475	21	21.5	0.1982	0.0676	0.0993	1.4689	0.5010	2.9319	0.6808	
29	485	28									
30	500	28									
31	495	30									
June											
1	500	30									
2	480	30	17.4	0.1776	0.0650	0.0964	1.4831	0.5428	2.7323	0.6743	
3	475	30									
4	470	29									
5	435	30									
6	415	16									
7	390	2	35.5	0.1723	0.0543	0.0922	1.4185	0.5351	2.6662	0.7049	

No. 77. TABLE V.

Date	Body wt.	Amt. of diet ingested	Loss of body wt.	Total fatty acid	Phospho-lipin	Cho-lesterol	Cholesterol / Phospholipin	Cholesterol / Total fatty acid	Total fatty acid / Phospholipin	Phospholipin / Cholesterol	Remarks
May	gm	gm	%	%	%						
15	480	29									Loosenes of teeth. Hemorrhages of caecum and right limb joint.
26	475	28	1.04	0.1291	0.0495	0.0792	1.6000	0.6135	2.6081	0.625	
27	470	29									
28	470	29									
29	465	30	3.12	0.1606	0.0619	0.0985	0.5913	0.6133	2.5945	0.6284	
30	460	30									
31	460	30									
June											
1	455	30	5.23	0.1624	0.0624	0.0999	1.6009	0.6151	2.6026	0.6266	Fed with scorbutic diet from 2/VI.
2	460	30									
3	445	30									
4	450	30									
5	445	30									
6	440	30									
7	435	30	9.39	0.1813	0.0653	0.1012	1.5927	0.5361	2.7764	0.6453	
8	435	30									
9	425	30									
10	420	30									
11	415	30									
12	400	20									
13	390	18	16.66	0.1754	0.0600	0.0874	1.4536	0.4983	2.9233	0.6865	
14	390	19									
15	395	17									
16	395	23									
17	400	22									
18	390	17									
19	390	21									
20	385	19									
21	385	30									
22	385	26									
23	380	25									
24	370	27	22.92	0.1624	0.0509	0.0722	1.4185	0.4446	3.1906	0.7049	

TABLE VI.

Date	Body wt.	Amt. of diet ingested	Loss of body wt.	Total fatty acid	Phospho-lipin	Cho-lesterol	Cholesterol / Phospholipin	Cholesterol / Total fatty acid	Total fatty acid / Phospholipin	Phospholipin / Cholesterol	Remarks
June	gm	gm	%	%	%	%					
2	715	6									
3	710	11	0.68	0.1574	0.0598	0.0953	1.5936	0.6054	2.6321	0.6285	Looseness of teeth, Hemorrhages of cæcum and right limb joint.
4	705	14									
5	710	18									
6	700	15	2.09	0.1589	0.0604	0.0939	1.5911	0.6098	2.6053	0.6279	
7	705	18									
8	700	20									
9	700	24	2.09	0.1689	0.0645	0.1034	1.6003	0.6122	2.6025	0.6237	
10	695	28									Fed with scorbutic diet from 10/VI.
11	690	23									
12	680	27									
13	680	26									
14	675	28									
15	680	28									
16	665	27	6.8	0.2528	0.0684	0.1019	1.4898	0.4031	3.6959	0.6712	
17	655	26									
18	650	28									
19	645	27									
20	640	26									
21	635	28									
22	635	30									
23	630	25	11.8	0.2256	0.0325	0.0986	1.5726	0.4326	3.6981	0.6358	
24	620	26									
25	615	25									
26	610	14									
27	585	10									
28	555	5									
29	530	9									
30	510	8	28.67	0.1°15	0.0515	0.0744	1.4447	0.7330	1.9709	0.6922	

No. 79. TABLE VII.

Date	Body wt.	Amt. of diet ingested	Loss of body wt.	Total fatty acid	Phospho-lipin	Cho-lesterol	Cholesterol / Phospholipin	Cholesterol / Total fatty acid	Total fatty acid / Phospholipin	Phospholipin / Cholesterol	Remarks
June	gm	gm	%	%	%	%					
3	625	13									Looseness of teeth. Enlargement and hemorrhages of rib junction and right limb joint. Bloody stools.
4	605	8	4.72	0.1632	0.0624	0.0995	1.5961	0.6097	2.6154	0.6271	
5	590	5									
6	570	1									
7	545	8	14.3	0.1953	0.0754	0.1198	1.589	0.6132	2.5902	0.6293	
8	550	15									
9	555	19									
10	560	25	11.8	0.1842	0.0707	0.1123	1.5884	0.6097	2.6054	0.6295	Fed with scorbutic diet from 11/VI.
11	555	28									
12	565	26									
13	555	23									
14	570	22									
15	570	25									
16	570	28									
17	560	28	11.8	0.2048	0.0337	0.0942	1.4787	0.4599	3.2135	0.6762	
18	560	28									
19	565	26									
20	565	27									
21	560	22									
22	555	21									
23	550	22									
24	540	20	14.6	0.1824	0.0754	0.1082	1.4350	0.5932	2.4191	0.6969	
25	520	16									
26	505	18									
27	480	12									
28	460	8									
29	445	3									
30	425	5									
July											
1	410	5	34.45	0.1215	0.0376	0.0864	1.2781	0.7111	1.7973	0.7824	

No. 81.

TABLE VIII.

Date	Body wt.	Amt. of diet ingested	Loss of body wt.	Total fatty acid	Phospho-lipin	Cho-lesterol	Cholesterol / Phospholipin	Cholesterol / Total fatty acid	Total fatty acid / Phospholipin	Phospholipin / Cholesterol	Remarks
June	gm	gm	%	%	%	%					
14	495	5		0.1236	0.0473	0.0758	1.6025	0.6132	2.6131	0.6240	Looseness of teeth. Enlargement and hemorrhages of caecum.
15	490	5									
16	470	7									
17	455	12	8.08	0.1658	0.0637	0.1015	1.5934	0.6122	2.6028	0.6276	
18	455	11									
19	450	9									
20	435	10	12.1	0.1704	0.0655	0.1039	1.5862	0.6097	2,6015	0.6304	Fed with scorbutic diet from 21/VI.
21	455	14									
22	470	17									
23	470	16									
24	470	15									
25	470	13									
26	470	15	5.05	0.2064	0.0699	0.1069	1.5293	0.5131	2.9585	0.6538	
27	470	15									
28	465	12									
29	450	16									
30	445	14									
July											
1	435	14									
2	425	14	14.1	0.1986	0.0606	0.0983	1.6221	0.4949	3.2772	0.6165	
3	420	13									
4	400	10									
5	390	8									
6	360	8									
7	340	10									
8	310	5	35.3	0.1854	0.0565	0.0809	1.4336	0.4364	3.2814	0.6981	

No. 85. TABLE IX.

Date	Body wt.	Amt. of diet ingested	Loss of body wt.	Total fatty acid	Phospho-lipin	Cho-lesterol	Cholesterol / Phospholipin	Cholesterol / Total fatty acid	Total fatty acid / Phospholipin	Phospholipin / Cholesterol	Remarks
June	gm	gm	%	%	%	%					
19	665	3		0.1662	0.0641	0.1025	1.5991	0.6167	2.5928	0.6254	Looseness of teeth. Enlargement and hemorrhages of rib junction.
20	635	5									
21	620	6									
22	585	7	12.03	0.1677	0.0636	0.1014	1.5943	0.6047	2.6367	0.6272	Hemorrhages of the joint of lower limb.
23	580	8									
24	605	23									
25	595	16	10.52	0.1600	0.0616	0.0985	1.5990	0.6156	2.5974	0.6255	Fed with scorbutic from 26/VI.
26	600	20									
27	615	25									
28	605	22									
29	600	25									
30	595	23									
July											
1	575	15									
2	565	13	15.03	0.2014	0.0675	0.1058	1.5674	0.5253	2.9837	0 6380	
3	565	13									
4	565	14									
5	555	7									
6	555	11									
7	545	14									
8	520	6									
9	495	3	25.5	0.2629	0.0800	0.1198	1.4975	0.4557	3.2863	0.6678	
10	470	5									
11	445	4									
12	440	3	33.8	0.1640	0.0642	0.0949	1.4626	0.5780	2.5545	0.6765	

No. 90.

TABLE X.

Date	Body wt.	Amt. of diet ingested	Loss of body wt.	Total fatty acid	Phospho-lipin	Cho-lesterol	Cholesterol / Phospholipin	Cholesterol / Total fatty acid	Total fatty acid / Phospholipin	Phospholipin / Cholesterol	Remarks
June	gm	gm	%	%	%	%					
19	635	18									
20	620	14	2.35	0.1708	0.0656	0.1053	1.6052	0.6165	2.6037	0.6229	Looseness of teeth. Enlargement and hemorrhages of rib junction.
21	610	14									
22	605	12									
23	610	12	3.95	0.1705	0.0670	0.1052	1.5701	0.6170	2.5448	0.6369	Hemorrhages of caecum and joint.
24	595	8									
25	590	17									
26	605	16	4.70	0.1762	0.0676	0.1075	1.5902	0.6101	2.6065	0.6288	Fed with scorbutic diet from 27/VI.
27	605	11									
28	605	15									
29	590	13									
30	585	17									
July											
1	585	13									
2	570	20	10.2	0.2428	0.0733	0.1044	1.4243	0.4299	3.3124	0.7021	
3	570	22									
4	565	12									
5	540	5									
6	520	9									
7	500	8									
8	470	5	25.9	0.2375	0.078	0.1123	1.4397	0.4729	3.0449	0.6945	
9	445	1									
10	420	0	33.7	0.2143	0.0647	0.0924	1.4281	0.4312	3.3122	0.7002	

TABLE XI

		Total fatty acid	Phospho-lipin	Cholesterol	Phospholipin / Cholesterol	Cholesterol / Total fatty acid	Total fatty acid / Phospholipin	Phospholipin / Cholesterol
		%	%	%				
Normal period	1st blood specimen	0.1520	0.0683	0.0934	1.607	0.614	2.608	0.624
	2nd „	0.1670	0.0642	0.1021	1.590	0.614	2.599	0.628
	3rd „	0.1722	0.0661	0.1057	1.598	0.615	2.604	0.624
	Average	0.1637	0.0629	0.1004	1.598	0.614	2.604	0.626
Scorbut. period	1st blood specimen	0.2082	0.0699	0.1059	1.517	0.513	2.985	0.660
	2nd „	0.2060	0.0685	0.1027	1.502	0.502	3.024	0.667
	3rd „	0.1613	0.0584	0.0846	1.420	0.545	2.730	0.703
	Average	0.1918	0.0656	0.0977	1.479	0.520	2.913	0.677
*Difference in %		+17.1	+4.3	−2.7	−17.4	−15.3	+11.9	+8.2

* It indicates the difference in which an average value in scorbutic period is compared with that in normal.

change in the cholecterol content of the blood in scurvy. In our experiment, however, there is shown some decrease just at the end of the scorbutic period.

The rate of $\frac{\text{cholesterol}}{\text{phospholipin}}$ in the blood in normal period is averaged by 1.598 while that in the scorbutic is 1.479 on an average; namely the latter shows an inclination towards a slight decrease. This is due to the fact that the rate of decrease of cholesterol content in the blood becomes greater than that of phospholipin at the end of the scorbutic period.

The rate of $\frac{\text{cholesterol}}{\text{total fatty acid}}$ in the blood in normal period is almost constant in guinea pigs and the average value is 0.614 while in the scorbutic it is averaged by 0.520. There is seen a tolerable decrease of the rate in the latter and it is chiefly due to the increase of total fatty acid in the blood of scorbutic animals.

$\frac{\text{Total fatty acid}}{\text{Phospholipin}}$ is averaged by 2.604 in normal and 2.913 in scurvy. In this case, the latter shows rather an increase in a slight degree.

$\frac{\text{Phospholipin}}{\text{Cholesterol}}$ is 0.626 and 0.677 in respective normal and scorbutic periods. It is slightly high in the latter; especially at the end of scurvy there has been shown a maximal value, 0.703. This is due chiefly to the fact that the cholesterol content in the blood of scorbutic animals decreases remarkably at that period.

Summary.

1. The content of total fatty acid in the blood of scorbutic guinea pigs is always larger than that of the normal. When the body weight of scorbutic animals begins to diminish, it shows a notable increase and finally at the advanced stage of scurvy, again it decreases, yet it remains still larger than the value at the beginning of normal period.

2. The phospholipin content of the blood in scorbutic period runs almost parallel to the content of total fatty acid in the period.

Even at the end of scurvy, it remains still above the initial value in normal period.

3. Cholesterol content of the blood in scorbutic period does not differ from that in the normal, with exception of the end period of scurvy in which there is, in most cases, seen a tolerable decrease.

4. The rate of cholesterol to total fatty acid in normal and in scorbutic period is 0.614 and 0.520 on an average respectively. The diminution of the latter is approximately 15%.

5. The rate of phospholipin to cholesterol in normal and scorbutic is 0.626 and 0.677 on an average respectively. The increased rate of the latter is 8.2%.

6. The rate of cholesterol to phospholipin is 1.598 in normal while that in scorbutic period is 1.479, i. e., in the latter there is a decrease of 7.4%.

7. The rate of total fatty acid to phospholipin is 2.604 in normal and 2.913 in the scorbutic period, i. e., 11.9% in the latter is increased.

Conclusions.

1. The decrease of $\frac{\text{Cholesterol}}{\text{Total fatty acid}}$ in the blood is suggested as a quantitative criterion in C-vitamin investigations on guinea pigs.

2. There may occur some disturbances of fat metabolism in the guinea pigs fed on a vitamin C free diet.

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STUDIES IN EXPERIMENTAL SCURVY.

XV. Changes in the Blood of Guinea Pigs Fed on a Vitamin C Free Diet.

By

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I. Introduction.

It is a well known fact that Barlow's disease and experimental scurvy are caused by a deficiency of vitamin C in the diet and that hemorrhagic diathesis and anaemia in the animal prevail. For this reason, the changes in the blood of the animals suffering from the deficiency disease have been studied by many investigators. Bierich (1919) suggested that the essential lesion in human scurvy, as in the experimental one, is an interference with the nutrition of the capillary endothelium. Philip Wales drew attention to the reduction in the number of red corpuscles. Wasserman (1918) and Shattack (1928) have also reported a secondary anaemia with a relative lymphocytosis. The former has found that the red corpuscles are sometimes decreased under 2,000,000 per cmm blood and that they are sometimes increased, especially during convalescence of the disease to 7,000,000 per cmm blood. On the other hand, Hess and Fish (1914), and Brandt (1919) stated that there is very little decrease in the the number of red cells in human scurvy while Mether and his coworkers (1930) described that the hemoglobin content is remarkably reduced in scurvy. Meyer and McCormick (1928) have also reported that there can be seen an anaemia induced by the characteristic decrease of red blood corpuscles and hemoglobin. The actual cause of the anaemia in scurvy has been variously interpreted. According to Hojer (1924), it is due to insufficiency

of red cell production. Mether and his coworkers (1930) attribute it to the disturbance of the function of the bone marrow in scurvy. Findlay (1921) has reported a decrease in the red blood corpuscles and the hemoglobin of the blood from the heart of scorbutic guinea pigs while he found that there is no appreciable change in those of the capillary blood. On the other hand, Gasperi (1926) believed that the decrease of red blood corpuscles in scurvy is temporary while Hryniewicz and Lawrynowicz (1927) have found that there is practically no change in their number. Nishi (1925) has found a decrease of the red cells and hemoglobin in the blood of patients suffering from Barlow's disease. Shattuck (1928) has reported that there is no change in the number of blood platelets in scurvy while Mether (1930) states that they show a slight increase. As for the clotting time of blood in scurvy, Findlay (1921) has confirmed MacRae's results (1908) that there is no diminution of the coagulation time. Nowodworski (1928) has also reached the same conclusion as is mentioned above. Nishi (1925) has reported that the amount of serum proteine is somewhat decreased. Although there are a number of papers on the blood changes in scurvy, they are mostly fragmentary. We have, therefore, performed a series of experiments on the blood changes such as hemoglobin content, blood coagulation time, water content, viscosity of blood, the content of fibrinogen and serum proteins, number of red cells and blood platelets, amounts of cephalin and thrombin in the experimental scurvy.

II. The Material for the Experiment.

Healthy guinea pigs, weighing from 400 to 550 gm were used for the present work. These animals were reared with Sherman's vitamin C free diet as base. To the control and scorbutic animals, fresh or boiled radish juice was given respectively. Under these rations, the latter showed scorbutic sympatoms after 2 or 3 weeks.

III. Experimental Procedures.

A. *Drawing blood.*

The blood was drawn by the same manner as is described in

previous paper. The hemoglobin and the blood-coagulation time were determined at the same time with the blood drawn from the different parts of the ear-lobe. The water content, viscosity, fibrinogen, and serum proteins were determined as a group. For the determination of the water content, the red cells and the platelets, the blood was drawn from the ear-lobe; for the viscosity and thrombin from the carotid artery and for the cephalin from the heart.

B. Hemoglobin.

Sahli's hemometer was applied for the determination of hemoglobin, and it was always done with the blood drawn at first.

C. Blood-coagulation Time.

For the present work, Sato's modification of Biffi-Brock's coagulometer was employed. The measuring was always done with the blood newly drawn from the ear-lobe. When the blood was repeatedly drawn from the same part of the ear vein, the coagulation time would be gradually shortened. The minimum coagulation time was taken instead of the maximum one to get an accurate result.

D. Water content of blood.

About 100 mg. of the blood are transferred to a small weighed piece of dried absorbent paper, about 16×28 mm in size. It is immediately weighed again. The paper is placed in the drying oven at 100° for one hour. When convenient it is cooled and again weighed. The water content of the blood is calculated from the loss of weight.

E. Viscosity.

Ostwald viscosimeter was applied for the determination of viscosity of the blood.

F. Fibrinogen and Serum proteins.

For the determination of fibrinogen and serum proteins in the blood, Starlinger's refractometer was used.

G. Red blood corpuscles.

The number of red cells was counted by Thoma Zeiss hemacytometer.

H. Blood platelets.

Fonio's method (1912) was employed for the counting of the blood platelets.

I. Thrombin.

The determination of thrombin in the serum was done by Wohlgemuth's method (1910).

J. Cephalin.

The cephalin content of the blood was estimated by Nakamura's nephelometric method (1927). The cephalin used as a standard was prepared from the brain of calf.

IV. Results of Experiment.

The hemoglobin content was estimated with the guinea pig's three times at two days intervals in normal period and at four or five days intervals in scorbutic period.

According to the results, as is shown in Table I, there can be seen some decrease of hemoglobin content in the blood of

Table I. Hemoglobin.

No. of animal	Body wt.		Normal period			Scorbutic period		
	Initial	Final	1st	2nd	3rd	4th	5th	6th
	gm	gm						
69	540	350	81	83	85	88	90	82
70	500	305	73	78	80	83	85	72
74	500	370	77	80	84	85	70	67
75	605	390	74	78	80	83	67	65
77	510	370	78	80	80	80	73	68
78	710	510	85	87	86	73	70	70
79	605	410	76	88	84	78	68	65
81	520	310	78	82	84	75	60	50
85	665	440	78	77	75	75	70	68
90	635	420	80	80	78	72	70	68
Maxim.	710	510	85	88	86	88	90	82
Minim.	500	305	73	77	75	72	60	50
Average	579	388	78	81	82	79	72	68

scorbutic guinea pigs. It is especially remarkable at the end of scurvy, i. e., it shows 68.0 while the average value in normal period is 80.3.

The average clotting time or normal and scorbutic periods as is seen in Table II, was 4′47″ and 5′50″ respectively. It was shown that the coagulation time of the blood of scorbutic animals was prolonged by 1′03″ over that of the normal one.

Table II. Blood-coagulation time.

No. of animal	Body wt.		Normal period			Scorbutic period		
	Initial	Final	1st	2nd	3rd	4th	5th	6th
	gm	gm						
69	540	350	4′30″	4′30″	4′00″	5′00″	6′00″	6′00″
70	500	305	5′30″	5′30″	5′00″	5′30″	5′30″	6′30″
74	500	370	4′30″	4′30″	4′00″	6′00″	6′00″	6′30″
75	605	390	5′00″	4′30″	4′30″	5′30″	6′30″	7′00″
77	510	370	6′00″	5′30″	5′30″	5′30″	6′00″	6′30″
78	710	510	5′00″	4′30″	4′30″	6′00″	6′00″	6′00″
79	605	410	4′00″	3′30″	3′30″	4′00″	5′30″	5′30″
81	520	340	5′00″	4′30″	4′30″	5′30″	6′30″	6′30″
85	665	440	5′00″	5′00″	5′00″	5′00″	5′30″	5′30″
90	635	420	5′30″	5′30″	5′30″	5′00″	6′00″	6′30″
Maxim.	710	510	6′00″	5′30″	5′30″	6′00″	6′30″	7′00″
Minim.	500	305	4′00″	3′30″	3′30″	4′00″	5′30″	5′30″
Average	579	388	5′00″	4′45″	4′36″	5′18″	5′57″	6′15″

The content of the water, the fibrinogen, the serum proteins in the blood and the viscosity of the blood of normal and scorbutic animals are shown in Tables III a and b.

The water content of the blood of normal and scorbutic animals varies in 79.8–81.1% and 80.6–82.4% respectively. There can be seen a slight increase of the water content in the blood of scorbutic guinea pigs. The viscosity of the blood was 1.346 in maximum, 1.271 in minimum, and 1.305 on an average in normal period, while these values in scorbutic period were 1.269, 1.228 and 1.252 respectively. In the latter case, there could be seen a

Table III a. (Normal period)

No. of animal	Body wt.		Water content	Viscosity	Fibrinogen	Serum-protein
	Initial	Final				
	gm	gm	%		%	%
154	460	405	79.8	1.226	0.22	3.50
155	440	430	79.9	1.271	0.22	3.56
158	440	420	79.9	1.328	0.28	3.32
160	495	415	80.1	1.346	0.22	3.64
164	405	420	81.1	1.283	0.26	3.72
Maxim.	495	430	81.1	1.346	0.28	3.72
Minim.	405	405	79.8	1.271	0.22	3.32
Average	448	418	80.1	1.305	0.24	3.55

Table III b. (Scorbutic period)

No. of animal	Body wt.		Water content	Viscosity	Fibrinogen	Serum-protein	Scor-butic days
	Initial	Final					
	gm	gm	%		%	%	
148	400	270	82.1	1.228	0.16	3.12	16
151	545	420	81.0	1.262	0.19	3.64	15
153	490	395	82.4	1.269	0.20	3.32	16
156	455	350	81.8	1.244	0.20	3.42	17
157	445	295	80.6	1.255	0.22	3.54	16
Maxim.	545	420	82.4	1.269	0.22	3.64	17
Minim.	400	270	80.6	1.228	0.16	3.12	15
Average	467	346	81.6	1.252	0.19	3.41	16

slight decrease of viscosity. The amount of fibrinogen, on an average, was 0.24% in normal period and 0.19% in the scorbutic one. There was a decrease of 20.9% in the latter. On the other hand, the amount of serum proteins in normal and in scorbutic guinea pigs was 3.55% and 3.41% respectively. The difference between them was not remarkable.

As seen in Tables IV a and b, the number of red cells per cmm blood of normal guinea pigs was 6,512,000 in maximum, 5,880,000 in minimum and 6,134,400 on an average, while that of scorbutic ones at the end period was 5,096,000, 3,680,000 and 4,729,800

TABLE IV a. (Normal period)

No. of animal	Body wt.		Red blood corpuscles	Blood platelets	Thrombin	Cephalin
	Initial	Final				
	gm.	gm.	per cmm	per cmm	Ff	mg%
184	530	420	6,168,000	338,140	125.0	63.3
185	515	440	6,240,000	361,920	125.0	60.5
186	520	455	5,880,000	335,140	125.0	59.3
187	450	410	6,136,000	306,800	125.0	55.7
188	515	545	6,432,000	347,328	125.0	66.9
189	625	565	6,512,000	384,208	62.5	63.2
190	550	545	6,136,000	349,752	62.5	78.8
191	575	420	5,968,000	340,176	125.0	60.5
192	440	440	5,912,000	366,544	125.0	68.8
193	520	430	5,960,000	387,400	62.5	70.3
Maxim.	625	565	6,512,000	387,400	125.0	78.8
Minim.	440	410	5,880,000	306,800	62.5	55.7
Average	524	467	6,134,400	351,741	112.5	64.7

TABLE IV b. (Scorbutic period)

No. of animal	Body wt.		Red blood corpuscles	Blood platelets	Thrombin	Cephalin	Scorbutic days
	Initial	Final					
	mg.	mg.	per cmm	per cmm	Ff	mg%	
194	470	365	4,976,000	248,800	16.1	47.0	20
196	515	430	4,656,000	223,488	32.3	50.6	21
197	525	350	4,688,000	257,840	62.5	63.5	21
199	495	385	4,984,000	244,216	32.3	66.3	19
202	530	390	4,560,000	196,080	16.1	56.0	20
203	490	315	3,680,000	180,320	16.1	44.5	18
205	550	365	4,872,000	263,088	16.1	60.3	19
207	570	425	4,818,000	240,900	32.3	63.0	20
208	465	415	5,096,000	295,568	16.1	58.2	16
210	460	320	4,968,000	258,336	16.1	49.0	18
Maxim.	570	430	5,096,000	295,568	62.5	66.3	21
Minim.	460	315	3,680,000	180,320	16.1	44.5	16
Average	507	376	4,729,800	240,864	25.6	55.8	19

respectively. There was seen a tolerable decrease of blood red cells in scurvy. The number of blood platelets per cmm blood of normal animals was 387,400 in maximum, 306,800 in minimum, and 351,741 on an average, while that of the scorbutic ones at the end period was 295,568, 180,320 and 240,864 respectively. The rate of the decrease was found to be larger than that in blood red cells. That is to say, it was 31% in the former while it was 23% in the latter. The amount of thrombin in the serum of normal guinea pigs was, on an average, 112,5(Ff) while that of the scorbutic ones at the end period was reduced to 25.6(Ff). Cephalin content in the blood of scorbutic animals at the end period was also decreased in a slight degree, i. e., it was, on an average, 64.7 mg% in normal guinea pigs and 5.58 mg% in the scorbutic ones.

Conclusions.

From the results described above, the changes in blood of the guinea pigs in experimental scurvy was found to be a follows:

1. The hemoglobin content is reduced.
2. The blood-coagulation time is slightly prolonged.
3. The water content is somewhat increased.
4. The viscosity diminishes in a slight degree.
5. The fibrinogen content is somewhat decreased.
6. The amount of serum proteins is almost intact.
7. The blood red cells are remarkably decreased.
8. The blood platelets are also decreased, and the rate of the decrease is found to be larger than that in the blood red cells.
9. The thrombin content(Ff) in the serum is decreased.
10. The cephalin in the blood is also decreased to a slight extent.

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ÜBER DEN EINFLUSS DER GALLENSÄURE AUF DEN CALCIUMSTOFFWECHSEL. (V)

Die Calcium- und Phosphorsäureausscheidungen im Kot unter Zufuhr von Gallensäure und Adrenalin beim normalen sowie beim splanchnikotomierten Hund.

Von

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(Eingegangen am 3. Juni 1932)

Die Erdalkalienphosphate werden bekanntlich nicht nur im Harn und in der Galle, sondern auch, und zwar in der Hauptsache, durch den Darm im Kot ausgeschieden.

Da nun die Gallensäure einen enterohepatischen Kreislauf bildet, so muss die Ausscheidung der Erdalkalienphosphate durch den Darm durch die Gallensäure verscheiden beeinflusst werden, je nachdem sie im Organismus vermehrt oder vermindert wird. So hat Fuziwara (1931) den Einfluss der Cholsäure auf die Calciumausscheidung im Kot des Hundes untersucht und gefunden, dass die Kalkausscheidung im Kot durch subcutane Zufuhr von Cholsäure vermindert wird. Die Erdalkalienphosphate stammen nicht nur aus dem Darm her, sondern auch aus der Galle und der Nahrung.

In seiner vierten Mitteilung hat Fuziwara (1931) bereits darauf hingewiesen, dass die Kalkausscheidung im Kot stark von der Art der Nahrung abhängt. Andrerseits wurde von Kawada (1931) bewiesen, dass die Zufuhr von Gallensäure beim Hunde die Kalkausscheidung in der Gallen vermehrt. Doch wird die Kalkausscheidung im Kot durch Zufuhr von Gallensäure vermindert, da der aus der Leber ausgeschiedene Kalk wieder in der Gallenblase resorbiert und nur zum geringeren Teil in den Darm überführt werden kann, wie von Iwanaga (1917) nachgewiesen wurde.

Nach den Untersuchungen von Sekitoo (1929, 1930), Fuziwara (1931) und Kimura (1931) werden Calcium und Phosphorsäure im Harn und im Blut durch die Zufuhr von Gallensäure vermehrt, und die Gallensäure steht mit deren Stoffwechsel in innigem Zusammenhang. Andrerseits wurde von Sekitoo (1931) und Tsuji (1930) bewiesen, dass die Gallensäure auf den Sympathicus lähmend und gegen das den Sympathicus reizende Adrenalin antagonistisch wirkt. Also steht die Wirkung der Gallensäure in innigem Zusammenhang mit der Funktion des vegetativen Nervensystems. Im oben erwähnten Sinne habe ich den Einfluss der Gallensäure und des Adrenalin auf die Calcium- und Phosphorsäureausscheidung im Kot beim normalen und beim linksseitig splanchnikotomierten Hunde untersucht.

Experimenteller Teil.

Methode.

Beim Versuch wurden gut genährte, kräftige Hunde verwendet, die immer geformten Kot ausscheiden. Vor dem Versuch wurden sie wenigstens 2 Wochen lang mit folgender festgesetzter Nahrung gefüttert. Die tägliche Nahrung bestand aus Reis, getrockneten Fischchen oder Fleisch, Sojasuppe und Wasser, deren Mengenverhältnisse unten angegeben sind. Der Versuch und die Untersuchung des täglichen Kotes erfolgte in der von Fuziwara angegebenen Weise. So können drei Perioden unterschieden werden: die Zeit vor dem Versuch, der Versuch selbst, die Zeit nach dem Versuch.

In der Versuchsperiode wurden den normalen Hunden einerseits 0.5–2 ccm einer 1%igen Natriumcholatlösung subcutan oder intravenös, andrerseits 0,02 ccm einer 0,1%igen Adrenalinchloridlösung pro kg Körpergewicht morgens nach der Fütterung zu bestimmter Stunde subcutan verabreicht. Bei der anderen Gruppe von Hunden wurde linksseitige Splanchnikotomie nach Schulze (1900) ausgeführt. Nachdem die Hunde sich nach einer Woche von der Operationswunde erholt hatten, wurden sie zum Versuch verwendet.

Auch den splanchnikotomierten Hunden wurden dieselben Mengen von Natriumcholatlösung und von Adrenalinchloridlösung in der Versuchsperiode subcutan bezw. intravenös verabreicht, und der Calcium- und Phosphorsäuregehalt im Kot der 3 Perioden miteinander verglichen.

Die täglichen Fäces pro Periode wurden gewogen, mit Wasser breiartig gemacht und dann wieder gewogen. 1 g davon wurde in üblicher Weise verascht. Das Calcium der Asche wurde nach der modifizierten Methode von De Waard (1919, 1924) ausgefällt und mit Kaliumpermanganat titrimetrisch bestimmt. Die Phosphorsäure der Asche wurde nach der Neumannschen Methode bestimmt.

Nachdem der tägliche Calcium- und Phosphorsäuregehalt der Fäces sowohl der normalen als auch der splanchnikotomierten Hunde in der Vorperiode ungefähr konstant geworden, und zu gleicher Zeit an 2 Tagen Natriumcholatlösung oder Adrenalinchloridlösung subcutan bzw. intravenös injiziert worden war, um den Einfluss derselben auf den Gehalt an Calcium und Phosphorsäure im Kot zu prüfen, folgten nun zum abermaligen Vergleich 3–4 Tage, an denen keine Zufuhr stattfand. Die Resultate sind in den folgenden Tabellen zusammengestellt.

1. Zufuhr von Cholsäure beim normalen Hunde.

Aus den Versuchen 1–4 der Tabelle I und 1–2 der Tabelle II ergiebt sich, dass bei Fischnahrung der durchschnittliche tägliche Calciumgehalt der vorperiodischen Fäces 1,987 g, der der nachperiodischen Fäces 2,023 g und der der versuchsperiodischen 1,58 g beträgt, während bei Fleischnahrung der Calciumgehalt der vorperiodischen Fäces 0,301 g, der der nachperiodischen 0,260 g und der der versuchsperiodischen 0,22 g ist.

Durch Zufuhr von Cholsäure wird also der Calciumgehalt der Fäces im Vergleich mit dem der Vorperiode um 20,5–26,9% und, verglichen mit dem der Nachperiode, um 15,4–21,9% herabgesetzt. Auch der prozentuale Calciumgehalt wird in allen Fällen, sowohl bei Fisch- als auch bei Fleischnahrung, im Vergleich mit dem der Vor- und Nachperiode durch Zufuhr von Cholsäure herabgesetzt.

und zwar bei der ersteren um 22,1–32,8% und bei der letzteren um 17,1–19,6%. Die Kalkausscheidung im Kot wird durch die Art der Nahrung beeinflusst und durch die Zufuhr von Cholsäure herabgesetzt, wie Fuziwara (1931) in seinem Versuch bewiesen hat.

Was den Einfluss der Cholsäure und der Art der Nahrung auf die Phosphorausscheidung betrifft, so wird in den Versuchen 1–4 der Tabelle I und 1–2 der Tabelle II gezeigt, dass bei Fischnahrung die durchschnittliche tägliche Phosphorausscheidung der vorperiodischen Fäces (als P) 1,6845 g, die der nachperiodischen 1,748 g und die der versuchsperiodischen 1,3675 g beträgt, während bei Fleischnahrung die durchschnittliche tägliche Phosphorausscheidung der vorperiodischen Fäces (als P) 0,2758 g, die der nachperiodischen 0,2356 g, und die der versuchsperiodischen 0,1778 g beträgt.

Die tägliche Phosphorausscheidung im Kot wird durch Zufuhr von Cholsäure im Vergleich mit der vorperiodischen bei Fischnahrung durchschnittlich um 18,8%, bei Fleischnahrung um 35,5% vermindert, und im Vergleich mit der nachperiodischen bei Fischnahrung durchschnittlich um 21,7% und bei Fleischnahrung um 24,5% herabgesetzt. Der durchschnittliche prozentuale Phosphorgehalt wird ebenfalls durch Zufuhr von Cholsäure sowohl bei Fisch- als auch bei Fleischnahrung im Vergleich mit dem vorperiodischen und dem nachperiodischen herabgesetzt, und zwar wird er bei Fischnahrung durchschnittlich um 19,7% und 18,8% usd bei Fleischnahrung um 37,1 und 22,2% vermindert. Aus dem Ergebnisse geht hervor, dass die Phosphorausscheidung im Kot durch Zufuhr von Cholsäure herabgesetzt wird, wie das auch bei der Kalkausscheidung der Fall ist.

2 Zufuhr von Adrenalin bei normalen Hunden.

Bei diesem Versuch wurde den Hunden 0,02 ccm einer 0,1 %igen Adrenalinchloridlösung pro kg Körpergewicht in der Versuchsperiode subcutan verabreicht, und der Calcium- und Phosphorgehalt im Kot der 3 Perioden miteinander verglichen.

Aus den Versuchen 1–3 der Tabelle III ergibt sich, dass der

TABELLE I.

Versuch 1.

(Nahrung: 300 g Reis, 150 g getrocknete Fischchen, 30 ccm Sojasuppe und 1200 ccm Wasser)

Datum 1931	Körpergewicht kg	Kot (g) pro Tag	Ca g	Ca g durchsch. Wert	Ca %	Ca % durchsch. Wert	P g	P g durchsch. Wert	P %	P % durchsch. Wert	Bemerkungen
14/V	20.85	105	2.129	2.685	2.028	2.233	1.855	2.001	1.767	1.668	
15	21.00	103	2.465		2.393		1.732		1.681		
16	20.25	145	3.156		2.176		2.489		1.715		
17	19.65	128	2.988		2.334		1.930		1.508		
18	20.45	188	3.061	2.508	1.628	1.566	2.224	1.873	1.183	1.180	2 ccm Na-Cholatlös. (subcutan)
19	20.65	130	1.955		1.504		1.529		1.176		" " "
20	21.15	155	3.458	3.009	2.231	1.834	2.432	2.070	1.569	1.263	
21	21.45	175	3.318		1.896		2.192		1.253		
22	21.03	163	2.735		1.678		1.856		1.138		
23	20.75	165	2.524		1.530		1.801		1.091		

Versuch 2.

(Nahrung: 180 g Reis, 100 g getrocknete Fischchen, 20 ccm Sojasuppe und 1200 ccm Wasser)

26/VI	11.55	89	1.273	1.377	1.430	1.501	0.9785	1.0965	1.0995	1.2631	
27	11.70	81	1.188		1.466		1.0641		1.3137		
28	11.60	87	1.415		1.626		1.2296		1.4133		
29	11.50	84	1.243		1.480		1.1138		1.3260		
30	11.45	73	0.982	1.052	1.345	1.401	0.7859	0.8380	1.0766	1.1163	2 ccm Na-Cholatlös. (subcutan)
1/VII	11.45	77	1.122		1.457		0.8902		1.1560		" " "
2	11.50	75	1.287	1.418	1.716	1.683	1.0873	1.2388	1.4498	1.4854	
3	11.35	70	1.004		1.435		0.9953		1.4218		
4	11.40	88	1.346		1.529		1.0289		1.1692		
5	11.50	97	2.035		2.092		1.8438		1.9008		

Versuch 3.

(Nahrung: 180 g Reis, 100 g getrocknete Fischchen, 20 ccm Sojasuppe und 1200 ccm Wasser)

Datum 1931	Körpergewicht kg	Kot (g) pro Tag	Ca				P				Bemerkungen
			g	durchsch. Wert	%	durchsch. Wert	g	durchsch. Wert	%	durchsch. Wert	
8/VII	11.15	70	1.197	1.718	1.710	1.886	0.8367	1.5186	1.1955	1.6488	
9	10.85	88	1.831		2.080		1.6723		1.9003		
10	11.00	97	1.677		1.729		1.7402		1.7940		
11	11.00	107	2.166		2.024		1.8251		1.7056		
12	11.05	48	0.588	0.961	1.225	1.379	0.5528	0.9697	1.1517	1.3728	2 ccm Na-Cholatlös. (subcutan)
13	10.90	87	1.333		1.532		1.3866		1.5939		„ „ „
14	10.95	62	1.197	1.634	1.931	2.096	1.1084	1.6707	1.7877	1.9271	
15	11.00	77	1.627		2.114		1.6012		2.0795		
16	11.00	83	1.627		2.014		1.5175		1.8283		
17	10.85	92	2.037		2.215		1.8412		2.0130		

Versuch 4.

(Nahrung: 250 g Reis, 130 g getrocknete Fischen, 20 ccm Sojasuppe und 1200 ccm Wasser)

16/VII	24.25	100	2.2051	2.1686	2.205	2.212	2.1736	2.1222	2.1736	2.161	
17	24.00	88	1.9750		2.244		1.9640		2.2317		
18	24.10	90	1.9560		2.173		1.8614		2.0682		
19	23.90	114	2.5384		2.226		2.4898		2.1840		
20	23.95	101	1.7733	1.7980	1.756	1.755	1.7456	1.7912	1.7283	1.747	3 ccm Na-Cholatlös. (subcutan)
21	24.00	104	1.8247		1.755		1.8369		1.7662		„ „ „
22	23.80	97	2.0640	2.0291	2.128	2.011	2.0332	2.0118	2.0960	1.990	
23	23.80	92	1.8788		2.042		1.8756		2.0375		
24	23.90	117	2.3135		1.977		2.3896		2.0423		
25	23.95	98	1.8602		1.898		1.7490		1.7845		

Tabelle II.

Versuch 1.

(Nahrung: 300 g Reis, 150 g Fleisch, 30 ccm Sojasuppe und 1000 ccm Wasser)

Datum 1931	Körpergewicht kg	Kot (g) pro Tag	Ca g	Ca g durchsch. Wert	Ca %	Ca % durchsch. Wert	P g	P g durchsch. Wert	P %	P % durchsch. Wert	Bemerkung
2/VI	25.20	69	0.3652	0.3687	0.5293	0.5339	0.3976	0.3593	0.5762	0.5208	
3	25.10	85	0.5140		0.6400		0.5257		0.6185		
4	25.18	75	0.2990		0.3987		0.2585		0.3447		
5	25.05	47	0.2667		0.5674		0.2555		0.5436		
6	25.15	72	0.2670	0.2708	0.3153	0.3292	0.2218	0.2242	0.3081	0.2957	2 ccm Na-Cholatlös. (subcutan)
7	25.10	80	0.2745		0.3431		0.2266		0.2833		„ „ „
8	25.10	83	0.2574	0.3080	0.3101	0.4010	0.2527	0.2943	0.3045	0.3835	
9	25.15	79	0.4032		0.5104		0.4035		0.5108		
10	25.15	69	0.2778		0.4026		0.2816		0.4081		
11	25.10	77	0.2936		0.3810		0.2392		0.3106		

Versuch 2.

(Nahrung: 300 g Reis, 150 g Fleisch, 30 ccm Sojasuppe und 1000 ccm Wasser)

16/VI	17.00	57	0.2079	0.2334	0.3647	0.3922	0.1790	0.1924	0.3140	0.3163	
17	17.30	56	0.2614		0.4067		0.2103		0.3755		
18	17.05	59	0.2128		0.3606		0.1546		0.2620		
19	17.20	72	0.2714		0.3769		0.2258		0.3136		
20	16.95	56	0.1840	0.1687	0.3286	0.2921	0.1379	0.1315	0.2463	0.2273	3 ccm Na-Cholatlös. (subcutan)
21	17.00	60	0.1533		0.2555		0.1250		0.2083		„ „ „
22	17.00	70	0.2576	0.2123	0.3680	0.3490	0.2312	0.1769	0.3302	0.2931	
23	17.00	57	0.2070		0.3456		0.1665		0.2921		
24	17.00	61	0.2057		0.3372		0.1618		0.2652		
25	17.00	52	0.1802		0.3462		0.1481		0.2848		

durchschnittliche tägliche Calciumgehalt der vorperiodischen Fäces 2,161 g und der Phosphorgehalt derselben 1,6467 g, der Calciumgehalt der nachperiodischen Fäces 2,1054 g und der Phosphorgehalt derselben 1,6454 g beträgt, während bei den versuchsperiodischen Fäces der Calciumgehalt 2,5811 g und der Phosphorgehalt 1,9581 g beträgt.

Aus diesem Ergebnisse folgt, dass der tägliche durchschnittliche Calciumgehalt in der Versuchsperiode im Vergleich mit dem der Vorperiode um 16,3%, und im Vergleich mit dem der Nachperiode um 18,4% vermehrt ist, und dass der Phosphorgehalt im Vergleich mit dem der ersteren Periode um 15,9% und mit dem der letzteren Periode um 19,1% gesteigert ist.

Der durchschnittliche prozentuale Calcium- sowie Phosphorgehalt werden auch durch Zufuhr von Cholsäure im Vergleich mit dem der Vorperiode und dem der Nachperiode vermehrt, und zwar wird der Calciumgehalt verglichen mit dem der ersteren Periode um 11,9%, mit dem der letzteren Periode um 13,0%, und der Phosphorgehalt im Vergleich mit dem der ersteren Periode um 12,6%, mit dem der letzteren Periode um 12,6% vermehrt. Alle oben erwähnten Ergebnisse zeigen, dass die Kalk- sowie Phosphorausscheidungen im Kot durch Zufuhr von Adrenalin, sowohl absolut wie auch prozentual, gesteigert werden. Die Cholsäure wirkt also auch bei der Kalk- sowie Phosphorsäureausscheidung im Kot gegen das Adrenalin antagonistisch, wie es auch bei der Wirkung der Cholsäure auf die Glykogenbildung in der Leber und auf den Blutzucker der Fall war. Diese antagonistische Wirkung der Gallensäure auf das Adrenalin soll nach Miki (1932) und Tsuji mit der Funktion des vegetativen Nervensystems in innigem Zusammenhang stehen, indem die Gallensäure auf den Sympathicus lähmend wirkt. In diesem Sinne habe ich bei linksseitig splanchnikotomierten Hunden den Einfluss der Cholsäure und des Adrenalins auf die Kalk- sowie Phosphorsäureausscheidungen im Kot untersucht.

Tabelle III.

Versuch 1.

(Nahrung: 280 g Reis, 130 g getrocknete Fischchen, 30 ccm Sojasuppe und 1300 ccm Wasser)

Datum 1931	Körpergewicht kg	Kot (g) pro Tag	Ca				P				Bemerkungen
			g		%		g		%		
				durchsch. Wert		durchsch. Wert		durchsch. Wert		durchsch. Wert	
7/VIII	24.25	107	2.3408		2.188		1.8061		1.6880		
8	24.25	122	2.7056	2.4511	2.218	2.140	2.0718	1.8611	1.6982	1.6256	
9	24.25	130	2.6082		2.006		1.9529		1.5022		
10	24.10	100	2.1500		2.150		1.6138		1.6138		
11	24.05	123	2.8764	2.8953	2.338	2.426	2.1022	2.0676	1.7091	1.7308	Adrenalin (0.02 ccm pro kg)
12	24.20	116	2.9145		2.513		2.0329		1.7525		〃
13	24.30	112	2.1420		1.913		1.6828		1.5025		
14	24.25	112	2.2120	2.1472	1.975	1.995	1.7220	1.6548	1.5375	1.5364	
15	24.30	103	2.0511		1.991		1.5583		1.5364		
16	24.35	104	2.1840		2.100		1.6562		1.5925		

Versuch 2.

(Nahrung: 200 g Reis, 100 g getrocknete Fischchen, 20 ccm Sojasuppe und 1100 ccm Wasser)

Datum	Körpergewicht kg	Kot (g) pro Tag	Ca				P				Bemerkungen
			g		%		g		%		
				durchsch. Wert		durchsch. Wert		durchsch. Wert		durchsch. Wert	
7/IX	14.3	102	1.632	1.473	1.600	1.526	1.4307	1.2636	1.4027	1.3308	
8	14.5	92	1.391		1.512		1.2102		1.3155		
9	14.7	90	1.392		1.547		1.0986		1.2207		
10	14.7	95	1.475		1.447		1.3150		1.3842		
11	14.9	106	1.898	1.755	1.791	1.772	1.6016	1.4781	1.5110	1.4917	Adrenalin (0.02 ccm pro kg)
12	15.1	92	1.613		1.753		1.3545		1.4723		„ „ „
13	15.4	82	1.241	1.390	1.514	1.532	1.0197	1.1308	1.2435	1.2479	
14	15.3	100	1.569		1.569		1.3047		1.3047		
15	15.5	92	1.415		1.527		1.1224		1.2200		
16	15.5	88	1.334		1.516		1.0764		1.2232		

Versuch 3.

(Nahrung: 300 g Reis, 140 g getrocknete Fischchen, 30 ccm Sojasuppe und 1300 ccm Wasser)

18/IX	26.3	137	2.657	2.559	1.940	1.971	1.4256	1.8153	1.0409	1.4057	
19	25.8	113	2.220		1.965		1.7370		1.5372		
20	26.0	139	2.797		2.012		2.1712		1.5620		
21	25.5	130	2.560		1.930		1.9272		1.4825		
22	25.1	137	3.021	3.093	2.205	2.205	2.2087	2.3316	1.6771	1.6891	Adrenalin (0.02 ccm pro kg)
23	25.6	139	3.165		2.205		2.3644		1.7010		„ „ „
24	25.8	125	2.556	2.779	2.045	2.042	1.9771	2.1507	1.5817	1.5758	
25	26.0	129	2.704		2.093		2.0263		1.5708		
26	25.7	148	2.926		1.977		2.3693		1.6009		
27	25.3	143	2.930		2.048		2.2302		1.5596		

3. Bei Durchschneidung des linksseitigen Splanchnicusnervs.

Hierbei wurde der Einfluss der Splanchnicusdurchschneidung auf die Calcium- und Phosphorsäureausscheidung im Darm unter Fütterung mit bestimmten Nahrungen untersucht, indem der linke Splanchnicusnerv dicht oberhalb der Nebenniere durchgeschnitten wurde. Die Nahrung bestand aus 120 g Reis, 50 g getrockneten Fischchen, 20 ccm Sojasuppe und 800 ccm Wasser.

Aus den Versuchen 1–2 der Tabelle IV ist ersichtlich, dass der durchschnittliche tägliche Kalkgehalt der Fäces vor der Splanchniktomie der absoluten Menge nach 0,954 g, prozentual 2,133% beträgt, während er nach der Splanchnikotomie absolut 0,937 g, prozentual 2,092% beträgt.

Der durchschnittliche Phosphorgehalt der Fäces vor der Splanchnikotomie wird der absoluten Menge nach mit 0,8112 g und prozentual mit 1,826% angegeben, während er nach der Splanchnikotomie absolut 0,7896 g und prozentual 1,769% ist.

Die Kalkausscheidung in den Fäces wird also durch linksseitige Splanchniktomie der absoluten Menge nach um 1,8% und prozentual um 2% herabgesetzt. Auch die Phosphorausscheidung wird durch linksseitige Splanchnikotomie der absoluten Menge nach um 2,8% und prozentual um 3,2% herabgesetzt.

Obwohl der Verminderungsgrad der Ausscheidung von Kalk und Phosphor durch den Darm bei der linksseitigen Splanchnikotomie ganz schwach ist und innerhalb der Fehlergrenze des Versuches zu liegen scheint, so scheint mir doch die Verminderung der Kalk- sowie der Phosphorausscheidung durch die linksseitige Splanchnikotomie hervorgerufen zu sein, indem dadurch die Adrenalinsekretion der Nebenniere herabgesetzt wird, was aus dem Versuch von Tscheboksaroff (1911) hervorgeht.

Die Kalk- sowie Phosphorausscheidung durch den Darm wird durch Zufuhr von Adrenalin vermehrt, wie im zweiten Versuch gezeigt wurde. Nach dem Versuch von Okamura (1928) soll sich der Adrenalingehalt der Nebenniere durch Zufuhr von Gallensäure vermindern.

Tabelle IV.

Versuch 1.

(Nahrung: 120 g Reis, 50 g getrocknete Fischchen, 20 ccm Sojasuppe und 800 ccm Wasser)

Datum 1932	Körpergewicht kg	Kot (g) pro Tag	Ca g	Ca g durchsch. Wert	Ca %	Ca % durchsch. Wert	P g	P g durchsch. Wert	P %	P % durchsch. Wert	Bemerkungen
20/I	8,3	44	1.000	0.916	2.273	2.1806	0.9227	0.8327	2.097	1.987	
21	8.4	44	0.920		2.092		0.8564		1.946		
22	8.3	39	0.821		2.105		0.7637		1.983		
23	8.4	41	0.923		2.252		0.7880		1.922		
6/II	7.7	42	0.861	0.899	2.051	2.0840	0.7910	0.8087	1.883	1.881	27/I Operation
7	7.8	35	0.806		2.302		0.7064		2.018		
8	7.8	54	1.066		1.973		0.9393		1.795		
9	7.9	42	0.861		2.051		0.7681		1.829		

Versuch 2.

(Nahrung: 140 g Reis, 40 g getrocknete Fischchen, 20 ccm Sojasuppe und 800 ccm Wasser)

29/II	10.5	47	0.965	0.991	2.053	2.086	0.8055	0.7896	1.714	1.664	5/III Operation
1/III	10.5	47	1.053		2.240		0.8760		1.864		
2	10.3	48	0.952		1.983		0.7534		1.569		
3	10.4	48	0.993		2.068		0.7233		1.507		
11/III	10.0	48	1.034	0.975	2.153	2.099	0.8401	0.7704	1.750	1.658	
12	10.0	47	0.951		2.024		0.7312		1.555		
13	10.5	49	1.007		2.056		0.7913		1.615		
14	10.0	42	0.909		2.163		0.7191		1.712		

Die Kalk- sowie Phosphorausscheidungen beim splanchnikotomierten Hunde müssen also durch Zufuhr von Gallensäure noch mehr herabgesetzt werden, wenn die erste Herabsetzung von der Zufuhr von Adrenalin herrührt. In diesem Sinne habe ich den folgenden Versuch ausgeführt.

4 Zufuhr von Cholsäure bei linksseitig splanchnikotomierten Hunden.

Beim Versuch habe ich den Hunden nur den linken Nerv dicht oberhalb der Nebenniere durchschnitten, um die Ausfallserscheinung zu verstärken, weil der linke Splanchnicusnerv beide Nebennieren versorgt.

Aus den Versuchen 1–4 der Tabelle V erhellt, dass der durchschnittliche tägliche Kalk- sowie Phosphorgehalt in den vorperiodischen Fäces 1,315 g, bezw. 1,0744 g, der der Nachperiode 1,357 g bezw. 1,0702 g beträgt, während in den versuchsperiodischen Fäces der durchschnittliche tägliche Kalkgehalt 1,240 g und der Phosphorgehalt 1,0157 g ist.

Aus diesem Ergebnisse lässt sich ersehen, dass der tägliche Kalkgehalt in der Versuchsperiode sich im Vergleich mit dem der Vorperiode durchschnittlich um 5,7% und vergleichen mit dem der Nachperiode um 8,6% vermindert, und dass der tägliche Phosphorgehalt in der Versuchsperiode verglichen mit dem der ersteren Periode durchschnittlich um 5,5% und verglichen mit dem der letzteren Periode um 5,1% herabgesetzt wird. Der tägliche prozentuale Calcium- sowie Phosphorgehalt in der Versuchsperiode wird ebenfalls im Vergleich mit dem der Vorperiode und dem der Nachperiode vermindert, und zwar wird der Calciumgehalt verglichen mit dem der ersteren Periode um 3,5%, verglichen mit dem der letzteren Periode um 10,0% vermindert, und der Phosphorgehalt verglichen mit dem der Vorperiode um 1,6%, verglichen mit dem der Nachperiode um 6,0% herabgesetzt.

Diese verminderte Kalk- sowie Phosphorausscheidung im Kot bei Zufuhr von Cholsäure tritt beim normalen Hunde viel stärker auf als beim linksseitig splanchnikotomierten Hunde, wie in den Tabellen I, II und V zu sehen ist.

Aus diesem Ergebnisse lässt sich feststellen, dass die die Kalk- sowie die Phosphorausscheidung herabsetzende Wirkung der Cholsäure durch den Darm mit der Funktion des vegetativen Nervensystems eng verknüpft ist. Die die Kalk- sowie die Phosphorausscheidung im Darm herabsetzende Wirkung der Cholsäure wird durch den Ausfall des sympathischen Tonus zum Teil aufgehoben und tritt nur in schwächerem Grad auf. Aus den Ergebnissen der Versuche 3 und 4 geht hervor, dass die Kalk- sowie die Phosphorausscheidungen im Darm sowohl von der Adrenalinsekretion der Nebenniere als auch vom sympathischen Tonus abhängig sind, die bekanntlich beide in einem innigen Zusammenhang miteinander stehen. Weiter geht daraus hervor, dass diese Ausscheidungen auf direktem Wege durch den sympathischen Nerv mehr beeinflusst werden als auf dem Umwege über die Adrenalinsekretion.

5. *Zufuhr von Adrenalin bei splanchnikotomierten Hunden.*

Bei diesem Versuche wurden die beim zweiten Versuch den splanchnikotomierten Hunden in der Versuchsperiode 0,02 ccm einer 0,1%igen Adrenalinchloridlösung subcutan einverleibt.

Aus den Versuchen 1–2 der Tabelle VI ist zu ersehen, dass der durchschnittliche tägliche Kalkgehalt in den vorperiodischen Fäces 1,5597 g, der in den nachperiodischen 1,5307 g, und der in den versuchsperiodischen 1,6439 g beträgt. Der Phosphorgehalt der vorperiodischen Fäces beträgt 1,3125 g, der der nachperiodischen 1,2143 g und der der versuchsperiodischen 1,4018 g.

Aus diesem Ergebnisse folgt, dass der tägliche Kalkgehalt in den Fäces bei Zufuhr von Adrenalin im Vergleich mit dem der Vorperiode durchschnittlich um 5,5%, und verglichen mit dem der Nachperiode um 7,4% vermehrt wird, während der tägliche Phosphorgehalt in den Fäces bei Zufuhr von Adrenalin im Vergleich mit dem der Vorperiode durchschnittlich um 6,8%, und verglichen mit dem der Nachperiode um 15,4% vermehrt wird.

Was den durchschnittlichen prozentualen Calcium- sowie Phosphorgehalt in den Fäces bei Zufuhr von Adrenalin anbetrifft, so stellt sich heraus, dass beide im Vergleich mit der Vorperiode sowie

TABELLE V.

Versuch 1.

(Nahrung: 300 g Reis, 150 g getrocknete Fischchen, 30 ccm Sojasuppe und 1200 ccm Wasser)

Datum 1931	Körpergewicht kg	Kot (g) pro Tag	Ca				P				Bemerkungen
			g		%		g		%		
				durchsch. Wert		durchsch. Wert		durchsch. Wert		durchsch. Wert	
29/XI	25.0	98	2.399	2.375	2.448	2.254	1.900	1.843	1.939	1.764	9/XI Operation
30	24.8	101	2.448		2.424		1.810		1.792		
1/XII	24.9	111	2.371		2.140		2.008		1.809		
2	24.7	109	2.293		2.104		1.654		1.517		
3	24.5	102	2.203	2.188	2.160	2.093	1.688	1.644	1.655	1.575	0.5ccm Na-Cholatlös. (intravenös)
4	24.6	107	2.173		2.031		1.600		1.495		"
5	24.8	113	2.614	2.271	2.313	2.177	1.877	1.657	1.661	1.592	
6	24.7	115	2.479		2.156		1.779		1.547		
7	24.5	99	2.176		2.198		1.564		1.580		
8	24.6	89	1.815		2.039		1.407		1.581		

Versuch 2.

(Nahrung: 120 g Reis, 50 g getrocknete Fischchen, 20 ccm Sojasuppe und 800 ccm Wasser)

3/I	7.2	58	1.067	0.976	1.839	2.176	0.9057	0.8508	1.561	1.839	14/XII Operation
4	7.2	44	1.011		2.298		0.8935		2.030		
5	7.0	37	0.884		2,389		0.7078		2.010		
6	7.1	44	0.942		2.189		0.8244		1.874		
7	6.9	38	0.826	0.858	2.174	2.171	0.7685	0.8011	2.022	2.028	0,5ccm Na Cholatlös. (intravenös)
8	6.8	41	0.889		2.168		0.8337		2.033		"
9	7.0	36	0.936	0.976	2.600	2.469	0.8326	0.8449	2.313	2.148	
10	7.1	44	1.040		2.334		0.9008		2.045		
11	7.0	40	0.969		2.423		0.8158		2.040		
12	6.9	39	0.999		2.487		0.8305		2.194		

Versuch 3.

(Nahrung: 120 g Reis, 50 g getrocknete Fischchen, 20 ccm Sojasuppe und 800 ccm Wasser)

Datum 1931	Körpergewicht kg	Kot (g) pro Tag	Ca				P				Bemerkungen
			g		%		g		%		
				durchsch. Wert		durchsch. Wert		durchsch. Wert		durchsch. Wert	
7/II	7.8	35	0.806	0.933	2.302	2.127	0.7064	0.8334	2.018	1.894	27/I Operation
8	7.8	54	1.066		1.973		0.9693		1.795		
9	7.9	42	0.861		2.051		0.7681		1.829		
10	8.0	46	0.998		2.170		0.8899		1.933		
11	8.0	45	0.944	0.932	2.098	2.119	0.8045	0.8258	1.788	1.879	0,5ccm Na-Cholatlös. (intravenös)
12	8.0	43	0.920		2.140		0.8472		1.970		„
13	7.9	47	1.139	1.105	2.423	2.402	0.9651	1.0002	2.053	2.177	
14	7.9	43	1.033		2.403		0.9912		2.305		
15	7.8	47	1.139		2.423		1.0393		2.211		
16	7.8	47	1.109		2.359		1.0055		2.140		

Versuch 4.

(Nahrung: 140 g Reis, 40 g getrocknete Fischchen, 20 ccm Sojasuppe und 800 ccm Wasser)

11/III	10.0	48	1.034	0.975	2.153	2.099	0.8401	0.7704	1.750	1.658	5/III Operation
12	10.0	47	0.951		2.024		0.7312		1.555		
13	10.5	49	1.007		2.056		0.7913		1.615		
14	10.0	42	0.909		2.163		0.7191		1.712		
15	10.2	48	0.957	0.983	1.996	1.967	0.8175	0.7917	1.702	1.588	0,5ccm Na-Cholatlös. (intravenös)
16	10.0	52	1.008		1.938		0.7659		1.473		„
17	9.9	45	0.928	1.074	2.062	2.250	0.7022	0.7785	1.560	1.610	
18	9.5	49	1.076		2.195		0.7569		1.544		
19	9.6	46	1.183		2.571		0.8291		1.715		
20	9.5	51	1.109		2.174		0.8257		1.619		

TABELLE VI.

Versuch 1.

(Nahrung: 300 g Reis, 150 g getrocknete Fischchen, 30 ccm Sojasuppe und 1200 ccm Wasser)

Datum 1931	Körpergewicht kg	Kot (g) pro Tag	Ca				P				Bemerkungen
			g		%		g		%		
				durchsch. Wert		durchsch. Wert		durchsch. Wert		durchsch. Wert	
15/XI	25.5	120	2.335	2.261	1.945	1.859	2.016	1.874	1.680	1.543	9/XI Operation
16	25.5	125	2.460		1.968		2.017		1.614		
17	25.6	115	1.965		1.735		1.679		1.460		
18	25.7	126	2.254		1.789		1.784		1.416		
19	25.6	112	2.280	2.340	2.036	2.026	1.958	1.946	1.748	1.683	Adrenalin (1:1000) 0.2 ccm pro kg
20	25.7	119	2.400		2.017		1.933		1.624		„
21	25.8	100	1.931	2.132	1.931	1.974	1.488	1.646	1.488	1.533	
22	25.7	115	2.198		1.911		1.705		1.483		
23	25.9	107	2.117		1.979		1.629		1.522		
24	25.9	110	2.280		2.073		1.760		1.600		

Versuch 2.

(Nahrung: 100 g Reis, 50 g getrocknete Fischchen, 20 ccm Sojasuppe und 1000 ccm Wasser)

19/XII	7.30	37	0.8162	0.8583	2.205	2.307	0.6705	0.7510	1.8122	2.0135	14/XII Operation
20	7.35	36	0.8305		2.307		0.6673		1.8536		
21	7.40	39	0.9048		2.320		0.8293		2.1264		
22	7.25	37	0.8850		2.394		0.8368		2.2616		
23	7.25	38	0.9720	0.9477	2.558	2.434	0.8417	0.8575	2.2150	2.1990	0.02 ccm Adrenalin
24	7.25	40	0.9234		2.309		0.8327		2.1830		„
25	7.25	40	0.9163	0.9294	2.291	2.325	0.8097	0.7826	2.0243	1.9576	
26	7.30	40	0.9198		2.300		0.7760		1.9400		
27	7.28	39	0.9665		2.478		0.7938		2.0351		
28	7.23	41	0.9148		2.229		0.7510		1.8310		